



UNIVERSITI PUTRA MALAYSIA

**SELECTED HERBAL PLANTS AS GROWTH AND HEALTH
PROMOTERS IN BROILER CHICKENS**

**SEYED REZA HASHEMI
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**SELECTED HERBAL PLANTS AS GROWTH AND HEALTH PROMOTERS
IN BROILER CHICKENS**

By

SEYED REZA HASHEMI

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

December 2009



THIS THESIS IS DEDICATED TO

MY WIFE, MY PARENTS

WITH

LOVE AND GRATITUDE

AND ALSO TO

THE KIDS WHO HAVE THE ABILITY

BUT

DON'T HAVE FACILITIES AND EDUCATION



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Chairman: Zulkifli B Idrus, PhD

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A series of experiments were conducted to examine the effect of herbal supplementation as a growth and health promoters. An *in vitro* procedure was used to determine the antibacterial activity of the fruit of *Solanum torvum*, whole plant of *Euphorbia hirta*, and rhizomes of *Zingiber officinale*, *Curcuma longa* and *Zingiber zerumbet*. Among the five herbal plants studied only the aqueous extract of *Euphorbia hirta* showed considerable growth-inhibiting activity against *Staphylococcus aureus*.

Phytochemical screening and a toxicity evaluation of the selected plants was carried out in experiment II. Phytochemical screening study showed that selected plants contained volatile oils, tannins, alkaloids, saponins, flavonoids. Alkaloids and steroids were only detected in the aqueous extract of *Euphorbia hirta*. Toxicity evaluation also showed that based on liver enzyme and histopathology the herbal aqueous extracts were not toxic when administered 2,000 mg/kg body weight by the oral route.

Experiment III was conducted to examine the effects of dietary *Euphorbia hirta* supplementation on growth performance, nutrient digestibilities, digesta pH and ileal microbial population. 600 one-day-old broilers (Cobb-500) were housed in 30 pens of 20 birds each until day 42. The dietary treatments were: (1) basal diet (control), (2) basal diet + 0.02 g/kg virginiamycin (VM), (3) basal diet + 1.5 g/kg acidifier (OrgacidsTM) (OA), (4) basal diet + 2.5 g/kg *E. hirta* (EH2.5), (5) basal diet + 5 g/kg *E. hirta* (EH5.0) and (6) basal diet + 7.5 g/kg *E. hirta* (EH7.5). The higher levels of *E. hirta* inclusion (7.5 g/kg) improved FCR of broiler chickens at 42 days of age. The highest AMEn and protein digestibility were observed in EH7.5 group. On day 21, the total aerobic bacteria counts in the ileum content of the EH2.5, VM and control groups were significantly higher ($P < 0.05$) than the other groups. OA and VM supplementation significantly increased ($P < 0.05$) *Lactobacillus* count at day 21 and the lowest *Lactobacillus* count was noted in the EH7.5 birds. At 42 days, feeding of EH2.5 promoted greater numbers of *lactobacillus*, to compare with other groups and the lowest *Lactobacillus* count was enumerated in the control birds. On day 21, except for ileum and cecum, OA significantly reduced ($P < 0.05$) pH value. The trend in pH value of the gastrointestinal tract on day 42 and 21 was similar.

In experiment IV the effect of *E. hirta* and acidifier supplementation on gut colonization and organ invasion by *Salmonella enteritidis* was carried out. 120 day-old chicks were assigned to one of the three treatments: (1) basal diet (control), (2) basal diet + 7.5 g/kg *E. hirta* (EH), and (3) basal diet + 2 g/kg acidifier (OrgacidsTM) (OA). On day 3, each chick was inoculated orally with 1 mL of *S. enteritidis*



(1.5×10^8 cfu/bird). The control birds had significantly higher ($P < 0.05$) caecal *S. enteritidis* number than other groups following 7 and 14 days of challenge. Incidence of *S. enteritidis* colonization on the liver and spleen was significantly lower ($P < 0.05$) in OA birds following 7 days of challenge. The average of shedding time was longer in the control group than in the OA and EH7.5 treatments throughout the duration of the experiment.

Response of heat-stressed broiler chickens to *Zingiber zerumbet* and *Zingiber officinale* supplementation were investigated in the fifth experiment. A total of 300 male broiler chicks were randomly assigned to one of the following treatment. 1) Basal diet (control), 2) Basal diet +1% *Zingiber zerumbet* (ZZ1%), 3) Basal diet +2% *Zingiber zerumbet* (ZZ2%), 4) Basal diet +1% *Zingiber officinale* (ZO1%) and 5) Basal diet +2% *Zingiber officinale* (ZO2%). Diets were fed to the birds from day 28-42. At 35 days of age, half of the chickens were exposed to $38 \pm 1^\circ\text{C}$ for 2 h/d to another environmentally controlled chamber. Following 2 days of heat treatment heterophile/lymphocyte ratio and corticosterone were significantly elevated ($P < 0.05$). Significant feeding regimes \times heat treatment interaction was noted for serum glucose and potassium level ($P < 0.05$). Except for serum level of albumin, chloride, heterophile/lymphocyte ratio and plasma corticosterone seven days of heat treatment had no significant effect on blood parameters. Heat treatment markedly increases Hsp70 expression on day 42. Birds fed ZO2%, ZZ1% and ZZ2% had significantly greater ($P < 0.05$) Hsp70 response.

**TUMBUHAN HERBA YANG TERPILIH SEBAGAI PROMOTER
PERTUMBUHAN DAN KESIHATAN DALAM AYAM PEDAGING**

Oleh

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Satu siri eksperimen telah dijalankan untuk memeriksa kesan penambahan herba sebagai penggalak pertumbuhan dan kesihatan. Suatu prosedur *in vitro* telah digunakan untuk menentukan aktiviti anti-bakteria daripada buah *Solanum torvum*, pokok *Euphorbia hirta*, rizom-rizom *Zingiber officinale*, *Curcuma longa* dan *Zingiber zerumbet*. Di antara lima tumbuhan herba yang dikaji, hanya cecair ekstrak daripada *Euphorbia hirta* dapat merencatkan aktiviti pembiakan *Staphylococcus aureus*.

Semakan fitokimia dan suatu penilaian ketoksidan terhadap tumbuh-tumbuhan yang terpilih telah dijalankan dalam eksperimen II. Kajian semakan fitokimia telah menunjukkan bahawa tumbuh-tumbuhan yang terpilih mengandungi minyak-minyak teruap, tannin, alkaloid, saponin dan flavonoid. Hanya alkaloid dan steroid didapati dalam cecair ekstrak daripada *Euphorbia hirta*. Penilaian ketoksidan telah menunjukkan

bahawa ekstrak herba adalah tidak toksik semasa diberi melalui mulut pada 2,000 mg/kg berat badan berdasarkan kajian pada enzim hati dan histopatologi.

Eksperimen III telah dijalankan untuk memeriksa kesan-kesan penambahan *Euphorbia hirta* dalam diet terhadap prestasi pertumbuhan, pencernaan nutrien, pH hasil pencernaan dan populasi mikrobia dalam usus ileum. 600 ekor ayam pedaging jenis Cobb 500 telah dikaji selama 42 hari di dalam 30 sangkar di mana setiap sangkar sebanyak 20 ekor ayam. Enam rawatan diet adalah: satu kawalan dan lima diet yang mengandungi: (1) diet basal (kawalan), (2) diet basal + 0.02 g/kg virginiamycin (VM), (3) diet basal + 1.5 g/kg asid organik (OrgacidsTM) (OA), (4) diet basal + 2.5 g/kg *E. hirta* (EH2.5), (5) diet basal + 5 g/kg *E. hirta* (EH5.0) dan (6) diet basal + 7.5 g/kg *E. hirta* (EH7.5). Asid organik tersebut mengandungi asid-asid formik, fosforik, laktik, tartarik, sitrik, dan malik yang dihasilkan oleh Sunzen Corporation Sdn. Bhd. Malaysia. Kandungan *E. hirta* yang tertinggi (7.5 g/kg) dapat memperbaiki nisbah penukaran makanan dalam ayam-ayam pedaging pada umur 42 hari. AMEn dan pencernaan protein yang tertinggi dapat diperhatikan dalam kumpulan EH7.5. Pada umur 21 hari, jumlah bilangan bakteria aerobik dalam usus ileum yang terdapat dalam kumpulan EH2.5, VM dan kumpulan kawalan adalah signifikasinya lebih tinggi daripada kumpulan lain. Penambahan OA dan VM telah meningkatkan bilangan *Lactobasillus* dengan signifikan pada hari ke-21 dan bilangan *Lactobasillus* yang terendah adalah dicatatkan pada ayam EH7.5. Pada hari ke-42, pemberian kandungan makanan dengan EH2.5 telah menggalakkan penambahan bilangan *Lactobasillus* berbanding dengan kumpulan lain dan didapati bilangan *Lactobasillus* terlalu sedikit dalam ayam kawalan.

Pada hari ke-21, selain usus ileum dan sekum, OA dengan signifikan menurunkan nilai pH. Perubahan nilai pH dalam salur pencernaan adalah sama pada hari 42 dan 21. Dalam eksperimen IV kesan penambahan *E. hirta* dan asid organik terhadap pembentukan koloni dalam usus dan serangan organ oleh *Salmonella enteritidis* telah dijalankan. Anak-anak ayam telah dibahagikan kepada salah satu daripada tiga kumpulan: (1) diet basal (kawalan), (2) diet basal + 7.5 g/kg *E. hirta* (EH), dan (3) diet basal + 2 g/kg asid organik (OrgacidsTM) (OA). Asid organik mengandungi asid-asid formik, fosforus, laktik, tartarik, sitrik, dan malik yang dihasilkan oleh Sunzen Corporation Sdn. Bhd. Malaysia. Pada hari ke-3, setiap anak ayam telah diberi 1 mL *Salmonella enteritidis* (1.5×10^8 cfu/ekor) secara oral. Ayam-ayam kawalan mempunyai nombor *SE* dalam sekum yang signifikasinya lebih tinggi daripada kumpulan lain selepas 7 dan 14 hari dikaji. Keadaan pembentukan koloni *SE* dalam hati dan limpa adalah signifikasinya lebih rendah dalam ayam OA selepas 7 hari dikaji. Masa purata pembebasan adalah lebih lama dalam kumpulan kawalan berbanding kumpulan rawatan OA dan EH7.5 di sepanjang tempoh eksperimen.

Tindakbalas tekanan haba oleh ayam-ayam pedaging dengan penambahan *Zingiber zerumbet* dan *Zingiber officinale* telah dikaji dalam eksperimen kelima. Sebanyak 300 ekor ayam pedaging telah dikaji dengan diet-diet eksperimen telah diberi kepada ayam dari umur 28-42 hari. Lima rawatan diet adalah: 1) diet basal (kawalan), 2) diet basal + 1% *Zingiber zerumbet* (ZZ1%), 3) diet basal + 2% *Zingiber zerumbet* (ZZ2%), 4) diet basal + 1% *Zingiber officinale* (ZO1%), 5) diet basal + 2% *Zingiber officinale* (ZO2%). Pada umur 35 hari, separuh daripada ayam-ayam tersebut telah dikekalkan pada suhu



23°C manakala yang selebihnya telah didedahkan dengan suhu 38±1°C selama 2 jam/hari di tempat lain yang persekitarannya terkawal. Cara-cara pemakanan dan rawatan haba tidak mempunyai kesan yang signifikan pada kadar kematian di sepanjang tempoh kajian. Rawatan haba selama dua hari dengan signifikan telah meningkatkan nilai albumin, jumlah protein, kolesterol, glukose, sodium dan klorida dalam serum. Selepas 2 hari rawatan haba, nisbah H/L dan CS didapati meningkat dengan signifikan. Interaksi di antara cara-cara pemakanan x rawatan haba yang dicatatkan adalah signifikan bagi tahap glukosa dan potasium dalam serum. Selain nilai albumin dalam serum, klorida, nisbah H/L, dan kortikosteron dalam plasma, tujuh hari rawatan haba tidak memberikan kesan yang signifikan kepada parameter-parameter dalam darah. Rawatan haba telah meningkatkan pendedahan Hsp70 pada 42 hari. Ayam-ayam yang diberi ZO2%, ZZ1% dan ZZ2% mempunyai tindakbalas Hsp70 yang lebih banyak secara signifikan.



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I certify that a Thesis Examination Committee has met on 21 December 2009 to conduct the final examination of Seyed Reza Hashemi on his thesis entitled "Selected Herbal Plants as Growth and Health Promoters in Broiler Chickens " 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 (Phd).

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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 other institutions.

SEYED REZA HASHEMI

Date: 11 February 2010



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LIST OF ABBREVIATIONS

Abs	Antibodies
Ag	Antigens
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy nitrogen corrected
AP	<i>Andrographis paniculata</i>
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
BCA	Bicinchoninic acid
<i>B. cereus</i>	<i>Bacillus cereus</i>
BSA	Bovine Serum Albumin
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
BW	Body weight
Caco-2	Human colon carcinoma epithelial cell line
CDC	Center for Disease Control and Prevention
<i>C. longa</i>	<i>Curcuma longa</i>
CORT	Corticosterone
COX-2	Cyclooxygenase -2 (an enzyme responsible for inflammation)
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic
<i>E. hirta</i>	<i>Euphorbia hirta</i>
ELISA	Enzyme-Linked ImmunoSorbent Assay
EPEC	Enteropathogenic <i>E. coli</i>
EU	European Union
FAO	Food and Agriculture Organization
FDA	United States Food and Drug Administration
FEDESA	Fédération Européenne de la Santé Animale (European Federation of Animal Health)



FCR	Feed conversion ratio
FR	Feed Restriction
GE	Gross energy
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
GOT	Glutamine oxaloacetic transaminase
GPT	Glutamine pyruvic transaminase
HDL	High density lipoprotein
<i>H. influenzae</i>	<i>Haemophilus influenzae</i>
H/L ratio	Heterophile/Lymphocyte Ratio
HPLC	High Performance Liquid Chromatography
IC ₅₀	Median Inhibition Concentration (the concentration of substance that provides 50% inhibition to certain reaction)
IBS	Institute of Biosciences
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukins
iNOS	Inducible Nitric oxide synthases
LC ₅₀	Lethal Concentration 50 (concentration in water having 50% chance of causing death to aquatic life)
LDL	Low density lipoprotein
LD50	lethal dose of 50% of treated target animals
<i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>
MAP kinase	Mitogen-activated protein kinase
MBC	Minimum Bactericidal Concentration
ME	Metabolisable energy
MIC	Minimal inhibitory concentration
MR	Mortality rate
MRLs	Maximum residue limits
n	Number

