



UNIVERSITI PUTRA MALAYSIA

***COMPARATIVE EVALUATION OF THE MORPHOLOGY AND ENZYME
ACTIVITIES OF THE DIGESTIVE SYSTEM OF RED JUNGLE FOWL, AND
NATIVE VILLAGE AND COMMERCIAL BROILER CHICKENS***

KHALID KAMIL KADHIM

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KHALID KAMIL KADHIM

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**COMPARATIVE EVALUATION OF THE MORPHOLOGY AND ENZYME
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By

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August 2011

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Faculty: Veterinary Medicine

A study was carried out to compare the morphology of the digestive system, as well as the pancreatic and small intestinal enzymatics activities between the Red jungle fowl (RJF) and the native village chicken (NVC). The findings for the two breeds were compared with the commercial broilers (CBC) which had been consciously selected for high body weight. Ten male birds from each breed were serially euthanized at day 1, 10, 20, 56 and 120 post-hatch, respectively. The total body weight, as well as the absolute and relative weights of the digestive organs and the length of the intestinal segments were measured and recorded. Histological examinations of the digestive organs were carried out by light microscopy. The mucosubstances of the salivary glands and mucosa of the digestive tract were evaluated using histochemical methods. Histomorphometric evaluations of the intestinal mucosa, including the villus height, villus surface area and crypt depth, were also performed. In addition, the number of villi for each intestinal segment was counted through cross-sections of the villi obtained by a new method, and the intestinal surface area was also measured. The three-dimensional intestinal mucosa

was evaluated using scanning electron microscopy (SEM). The enzymatic activities of amylase, trypsin and chymotrypsin of the pancreas and small intestinal (duodenum, jejunum and ileum) contents were also investigated.

At day 10 post-hatch, the body weight of the CBC was higher ($P < 0.05$) than those of the NVC and RJF by approximately 4 and 6 folds, respectively, and these were increased to approximately 5 and 10 folds, respectively at day 20 post-hatch onwards. Nonetheless, the NVC showed a higher ($P < 0.05$) body weight than that of the RJF at approximately by two folds at day 20 post-hatch. The absolute weights and lengths of the digestive organs were dominantly greater ($P < 0.05$) in the CBC followed by the NVC, while the RJF had the least value. However, the results were reversed when the data attributed to the body weight, except for day 1 post-hatch, where the CBC showed the highest value. Grossly, the arrangement of the lingual papillae was in a straight row in the CBC and in a concave row in both the NVC and RJF, whereas, the pharyngeal papillae were double rows in the CBC, with a second rudimentary row in NVC and restricted to only a single row in the RJF. The shapes of the liver lobes in the RJF and NVC were more consistent with domestic chicken than CBC. As for the blood supply of the viscera, the numbers of the jejunal and ileal branches were found to be higher in the CBC compared to those in the NVC and RJF.

Histologically, the digestive system of the three chicken breeds showed many similarities. The proventricular glands are located in between the layers of their muscularis mucosa. The paneth cells and the enterochromaffin cells in the intestinal mucosa were investigated using the light microscopy of a specific staining method.

The histochemical observations of the salivary glands and mucosa of the digestive system for the presence of neutral and acid mucin (sulphated and carboxylated) revealed no difference between the breeds. The distribution of neutral and acid mucin was dominantly observed in the upper digestive tract, whereas, the intestinal mucin of the goblet cells contained mostly sulphated and carboxylated mucosubstances. The pancreatic ductal system showed the absence of the ductal glands and the goblet cells. However, the luminal mucin reaction of the epithelial cells lining these ducts was observed in all the three breeds.

The histomorphometric evaluations revealed that the thickness of the wall of the digestive organs was different between the three breeds. In absolute bases, the CBC showed dominantly greater values, followed by the NVC, while the RJF showed the least value. On the contrary, the data were reversed on the relative bases. The height and the surface area of the villi, as well as the muscularis externa were significantly higher ($P < 0.05$) in the CBC than those in the NVC and RJF. The number of villi in each intestinal segment was higher ($P < 0.05$) in the NVC and RJF compared to the CBC, and a similar result was also observed for the intestinal surface area, however, the jejunal surface area was higher in the CBC.

The SEM examinations revealed that the epithelial cells of the intestinal villi in the CBC have high activities at the early age, as demonstrated by the presence of numerous recesses, cell protuberance, cell clusters, dome-shaped cells, clear cell outlines, cell exfoliated and extrusion area. Whereas, in the NVC and RJF represented by the discontinuity of epithelia and corrugated surface. The characteristic star-shape villi in the mid-cecum region were observed in the three

breeds. The absolute and relative pancreatic enzyme activities were higher ($P < 0.05$) in the CBC than those of the NVC and RJF. Similar results were also observed for the absolute enzymatic activity of the intestinal contents. However, the data were shown to be reversed in the relative bases.

In conclusion, the difference in the growth rate between the breeds was positively associated with the growth and development of the digestive organs. In the CBC, changes markedly occurred during the early stages of life, whereby the change was observed to be rather biased towards the digestive tube giving the body organization designed for fast growth. During the late stages of development, it was biased towards the pectorals and the feathers. As for the NVC and RJF, it was obviously more valuable to have satisfactory protection against cold and rain at the earliest stage possible, and also some flying power in the young RJF as a protection against their predators. The effects of selection and the genetic variation might affect the growth rate of the digestive organs and the enzyme synthesis according to life cycle requirements, and this could be a limiting factor in the digestion and subsequently the growth rate.

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

**PENILAIAN PERBANDINGAN MORFOLOGI DAN AKTIVITI ENZIM
SISTEM PENCERNAAN AYAM HUTAN, DAN AYAM KAMPUNG DAN
AYAM PEDAGING KOMERSIAL**

Oleh

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Satu kajian telah dijalankan ini membezakan morfologi sistem pencernaan, pankreas dan aktiviti-aktiviti enzim pada usus kecil ayam hutan (RJF) dan ayam kampung (NVC). Penemuan kajian bagi kedua baka ayam ini akan dibandingkan dengan ayam pedaging komersial (CBC) terpilih yang mempunyai kadar pertumbuhan yang tinggi. Sepuluh ayam jantan bagi setiap baka dimatikan secara berkala pada hari ke 1, 10, 20, 56 and 120 paska tetasan. Jumlah berat badan keseluruhan dan kedua-dua berat mutlak dan relatif organ sistem pencernaan serta panjang segmen usus diukur dan direkod. Pemeriksaan histologi sistem ini dijalankan dengan menggunakan mikroskop cahaya. Bahan mukus pada kelenjar liur dan epitelium rembesan mukosa trakus pencernaan dikaji menggunakan teknik histokimia. Penilaian histomorfometrik mukosa usus merangkumi ketinggian vilus, kawasan permukaan vilus dan kedalaman kripta. Bilangan vili bagi setiap segmen juga dikira melalui keratan rentas vili menggunakan teknik baru dan luas permukaan usus juga diukur. Mukosa usus secara tiga dimensi telah dikaji menggunakan mikroskop elektron

imbasan. Aktiviti enzim amilase, tripsin dan kimotripsin pada pankreas dan kandungan usus kecil (duodenum, jejunum and ileum) juga diukur.

Penemuan kajian ini mendapati berat badan CBC lebih tinggi secara signifikan sebanyak lebih kurang 4 hingga 6 kali ganda daripada RJF dan NVC pada hari ke 10 pasca tetasan, dan bertambah sehingga 5 ke 10 kali ganda pada hari ke 20 pasca tetasan dan selepasnya. Berat mutlak dan panjang organ pencernaan adalah lebih tinggi pada CBC diikuti dengan NVC, sementara RJF mencatatkan bacaan terendah. Walau bagaimanapun, hasil kajian ini bertentangan bagi berat badan, kecuali pada hari pertama pasca tetasan, di mana CBC mencatatkan nilai tertinggi. Pemeriksaan mata kasar ke atas organ pencernaan mendapati susunan papila lingual adalah di dalam barisan lurus pada CBC dan barisan cengkung pada NVC dan RJF, sementara papila farinks tersusun dalam dua barisan pada CBC, barisan kedua hanya rudimen pada NVC dan pada RJF hanya terhad kepada satu baris sahaja. Bentuk lobul hati pada RJF dan NVC adalah lebih konsisten dengan ayam tempatan daripada CBC. Bagi bekalan salur darah visera, bilangan cabang jujenal dan ileal didapati lebih pada CBC daripada NVC dan RJF.

Secara histologi, sistem pencernaan ketiga baka ini menunjukkan banyak persamaan. Kelenjar proventrikular terletak di antara lapisan mukosa muskularis. Sel-sel Paneth dan enterokromafin pada mukosa usus dikaji menggunakan teknik pewarnaan khusus dan dilihat di bawah mikroskop cahaya. Tiada perbezaan ke atas pemerhatian histokimia bagi tindakbalas musin di kelenjar liur dan mukosa sistem pencernaan untuk kehadiran musin neutral dan berasid (sulfat dan karboksilat) pada kesemua baka ini. Taburan musin neutral dan berasid dapat dilihat mendominasi trakus

pencernaan hadapan, di mana musin sel goblet pada usus kebanyakannya mengandungi bahan mukus sulfat dan karboksilat. Sistem duktus pankreas tidak mempunyai kelenjar duktus dan sel goblet. Walau bagaimanapun, reaksi musin lumen pada sel epitelium yang melapisi duktus tersebut terdapat pada kesemua baka.

Penilaian histomorfometrik mendapati terdapat perbezaan pada ketebalan dinding organ pencernaan di antara ketiga baka ini. CBC menunjukkan nilai mutlak asas yang ketara diikuti oleh NVC manakala RJF menunjukkan nilai terendah. Sebaliknya, data ini bertentangan bagi asas relatif. Ketinggian vilus, kawasan permukaan vilus dan muskularis eksterna adalah lebih tinggi ($P < 0.05$) dicatatkan pada CBC berbanding NVC dan RJF. NVC dan RJF mempunyai bilangan vili yang lebih tinggi bagi setiap segmen usus daripada CBC dan penemuan yang sama didapati pada keluasan permukaan usus kecuali pada bahagian jejunum di mana CBC mempunyai nilai yang lebih tinggi.

Pemeriksaan menggunakan mikroskop imbasan mendapati sel epitelium pada vili usus CBC adalah lebih aktif pada peringkat awal umurnya dan ini ditunjukkan dengan terdapatnya banyak lekukan, penonjolan sel, kelompok-kelompok sel, sel berbentuk kubah, rangka sel yang jelas, pengelupasan dan kawasan penyembuhan. Sebaliknya, epitelium yang terputus dan permukaan yang beralun didapati pada NVC dan RJF. Vili berbentuk bintang pada sekum tengah terdapat pada kesemua baka. Aktiviti mutlak dan relatif enzim pankreas adalah lebih tinggi ($P < 0.05$) pada CBC daripada NVC dan RJF. Penemuan yang sama didapati pada enzim mutlak kandungan usus. Walau bagaimanapun, data ini bertentangan bagi nilai relatif.

Kesimpulannya, perbezaan kadar tumbesaran di antara baka-baka ini berkait secara positif dengan pertumbuhan dan perkembangan organ pencernaannya. Pada CBC, perubahan ketara berlaku semasa peringkat awal jangkahayat, di mana perubahan menyebelahi kepada tiub pencernaan yang menjurus kepada pembentukan badan bertujuan bagi pertumbuhan yang cepat. Semasa akhir perkembangan, ianya menjurus kepada bahagian pektoral dan bulu pelepah. Ternyata, adalah lebih baik bagi NVC dan RJF memiliki perlindungan yang memuaskan dalam menentang kesejukan dan hujan pada peringkat awal dan berdaya untuk terbang bagi RJF muda sebagai perlindungan daripada pemangsa. Kesan pemilihan dan variasi genetik boleh mempengaruhi kadar pertumbuhan organ dan sintesis enzim menurut keperluan kitaran hidup dan ini mungkin merupakan faktor yang membatasi pencernaan dan seterusnya kadar pertumbuhan.

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I certify that a thesis Examination Committee has met on (3ed Aug. 2011) to conduct the final examination of (khaild kamil kadhim) on his thesis entitled “**COMPARATIVE EVALUATION OF THE MORPHOLOGY AND ENZYME ACTIVITIES OF THE DIGESTIVE SYSTEM OF RED JUNGLE FOWL, AND NATIVE VILLAGE AND COMMERCIAL 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 (Doctor of Philosophy).

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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 is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



KHALID KAMIL KADHIM

Date: 3 August 2011

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LIST OF ABBREVIATIONS

ACD	Apical cecal diverticula
ANOVA	Analysis of variance
BAEE	Benzoyl-L- Arginine Ethylester
BTEE	N-Benzoyl-L-Tyrosine Ethylester
CBC	Commercial broiler chicken
cm	Centimeter
CP	Crude protein
d 1	Day one
DO1	Day one old
g	Gram
GIT	Gastrointestinal tract
H & E	Haematoxylin and Eosin
L	Liter
M	Molecular solution
min	minute
ml	Milliliter
mM	Millimole
N	Normal solution
NBF	Neutral Buffered Formalin
nm	Nanometer
NVC	Native village chicken
PAS	Periodic acid-Schiff reaction
PBS	Phosphate buffered normal saline
RGF	Red jungle fowl
SD	Standard deviation
SEM	Scanning Electron Microscope
SPSS	Statistical Package for the Social Sciences
vol	volume
wt	weight

INTRODUCTION

The origins of the domestic chicken (*Gallus domesticus*) may be traced to the wild Red jungle fowl (*Gallus gallus*) or the sub-species (*Gallus gallus gallus*) found in the Indian subcontinent and South East Asia (Lee and Amin-Babjee, 1993; Fumihito *et al.*, 1994). There are four species of jungle fowls: Red jungle fowl (RJF) (*Gallus gallus*), Javanese green jungle fowl (*Gallus varius*), Indian grey jungle fowl (*Gallus sonneratii*) and the Ceylon jungle fowl (*Gallus lafayettii*) (Crawford, 2003). The Red jungle fowl (*Gallus gallus*) is a tropical member of the pheasant family and the direct ancestor of the domestic chicken. It was first raised in captivity at least 5,000 years ago in India, and the domesticated form has been bred all over the world to serve as a very productive food source for both meat and eggs; meanwhile some breeds have been specifically developed to produce eggs. The range of the true species stretches from northeast India (where the pure species has almost certainly been diluted with backcrosses from domestic breeds) eastwards across southern China and down to Malaysia and Indonesia (Collias and Saichuae, 1967). The Red jungle fowl (*Gallus gallus*) is easily recognised as the male is more brilliantly coloured than the female; its feathers are between golden red to metallic green with a dented fleshy crest, white ears and after the summer months, it develops an eclipsed plumage. The female is dull brown gold colour (Appendix A).

There are five recognised sub-species of the Red jungle fowl based on external features. The Indian Red jungle fowl (*Gallus gallus murghi*) is found in the North and Northeast India, adjacent to Nepal and Bangladesh. The Tonkinese Red jungle

fowl (*Gallus gallus jabouillei*) is found in central-south China and North Vietnam. The Cochin-Chinese Red jungle fowl (*Gallus gallus gallus*) is found in East Thailand through central and South Laos to central and South Vietnam. The fourth subspecies, the Javan Red jungle fowl (*Gallus gallus bankiva*), is found in South Sumatra, Java and Bali while the Burmese Red jungle fowl (*Gallus gallus spadiceus*) is found in central-south China, Myanmar, Thailand, north Sumatra and is the only subspecies occurring in Peninsular Malaysia (Nishida *et al.*, 1992). The Red jungle fowl differs from domestic chickens in all eight characteristics that differentiate most sensitively between the wild Red jungle fowl and the domestic chicken: small body size, slow growth rate, very small comb, tail held down rather than up, male hackles shed in an eclipse molt, charcoal rather than pale legs, crowing call cut off abruptly rather than trailing off in a follow-through, and taking flight by taking off and flying up at a steep angle rather than by a long horizontal taxing run (Jackson and Diamond, 1996). There are very limited studies dealing with this bird in general and the digestive system in particular.

Village chickens comprise the bulk of the poultry industry in many developing countries and play a vital role in many poor rural households. They provide scarce animal protein (in the form of meat and eggs) and can be sold/bartered to meet essential family needs such as medicine, clothes and school fees. They also provide manure and serve to control pests; very often they are used in traditional ceremonies. Village poultry are generally owned and managed by women and children and improving their production can provide the first step out of poverty for the rural poor (Coplanad and Alder, 2005; Riise *et al.*, 2005). The indigenous chicken (*Gallus gallus domesticus*), commonly found in Southeast Asia, is descended from the

Southeast Asia Red jungle fowl (*Gallus gallus spadiceus*), through natural mating and selection (Beebe, 1926; Collias and Saichuae, 1967). In Malaysia, the original Malayan fowls were widespread in villages before the arrival of Europeans in the late 1800s. The present Malaysian native chickens, commonly known as *ayam kampung* (village chickens), are the result of cross-breeding of the Red jungle fowl with mixed exotic domestic breeds brought in by Europeans, mainly the British (Azahan and Zahari, 1983). Such chickens are now considered indigenous and are widespread in all Southeast Asian countries. In general, the indigenous chickens are of small body size, with different colours of plumage, and of dual-purpose type, with variable body conformation and physical characteristics (Appendix A). The chicken's market weight of 1-1.5 kg is reached in 4 to 5 months. As in the other countries of Southeast Asia, these indigenous chickens have been reared extensively for generations, in almost every village and suburban area throughout Malaysia, in free-range or integrated farming systems (Aini, 1990). Future prospects for rearing the village chicken are believed to be good because its meat, which is perceived to be tasty and of higher quality than that of exotic breeds (Crawford, 2003), has been traditionally in high demand.

During the late 1940s, broiler breeds required 12 weeks to reach the live body weight of 1.8 kg (Appendix A). Four decades later this period had been reduced by half, with a reduction of essentially 1 day/year to reach this weight, showing no evidence of abatement (Gyles, 1989). Recently, Marchini *et al.* (2011) have reported that the male broiler chickens reached 2.47 kg at 42 days post-hatch. Accordingly, the first 2 weeks post-hatch have become progressively the major part of the life of a broiler. Tremendous progress had been made in the selection of broilers for

increased growth, feed conversion and carcass quality (Crawford, 2003; Schreiweis *et al.*, 2005). Genetic selection, heterosis, and improvements in husbandry, nutrition and health have contributed to an escalating rate of growth for meat-type chickens. Previous work has demonstrated that chicks have highly developed leg muscles at hatch (Anthony *et al.*, 1989). Because broilers are marketed much earlier than in the past, many previous studies concentrated on the development of the gastro-intestinal tract (GIT) during the post-hatching growth period to illustrate the changes occurring in this tract during selection for fast growth (Dror *et al.*, 1977; Lilja, 1983; Katanbaf *et al.*, 1988; Nitsan *et al.*, 1991a, b).

To my knowledge there is a dearth of information relating to the Red jungle fowl, particularly its digestive system which has not been studied. But it is known that this species is characterised by a slow growth rate compared to the indigenous village chicken, and considering that the Red jungle fowl is an ancestor of the domestic chicken, comparisons can be made with the native village chicken which is also characterised by a slow growth rate. Data can then be compared with that of the commercial broiler chickens that have been consciously selected for high body weight to illustrate the effects of the artificial selection on the anatomical traits of the digestive organs. Therefore, the main aim of this study was to describe the morphology and the growth patterns of the digestive organs and the digestive enzyme activities (amylase, trypsin and chymotrypsin) in the pancreas and the intestine.

Based on the hypothesis that the morphology and enzyme activity of the digestive system in the Red jungle fowl, native village chicken and commercial broiler chicken are different, this study was undertaken with the following objectives:

- To describe the morphological changes of the digestive system of three breeds of chicken which differ in growth rate.
- To determine the digestive enzyme activities in the pancreas and small intestinal contents of three breeds of chicken which differ in growth rate.
- To compare the morphology and enzyme activities of the digestive system of three breeds of chicken, which differ in growth rate.

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