EFFECT OF FEEDING *Cyperus rotundus* EXTRACT ON THE AMELIORATION OF HEAT STRESS IN BROILER

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I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of the dissertation has been previously or concurrently submitted for a degree at this or any other university.

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ABSTRACT

This study was conducted to investigate the effects of feeding *Cyperus rotundus* extract on the growth performance, blood profiles and lymphoid organs status of heat stressed broiler. A total of 24 Cobb broilers at 21-day-old were assigned to four treatment groups. Three treatment groups were; T1 (heat stress), T2 (Heat stress plus 1 ml/ kg body weight of ascorbic acid) and T3 (Heat stress plus 1 ml/kg body weight of *Cyperus rotundus* extract) while T0 served as the control group. Results showed there was a sudden decrease in the body weight gain (BWG) until 2 weeks of heat stress in all treatment groups and in control. But there was an increment ($p<0.05$) in BWG, feed intake (FI) along with efficient feed conversion at 3rd week in the *Cyperus rotundus* extract feeding group. Thus, this study suggested that administration of 1ml/ kg body weight of *Cyperus rotundus* extract can exert ramification on heat stressed broiler after 2 weeks of feeding only.

KESAN PEMEBERIAN EKSTRAK Cyperus rotundus TERHADAP PENAMBAHBAIKAN KADAR TEKANAN HABA AYAM PEDAGING

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesan pemberian ekstraksi rumpur halia hitam terhadap penambahbaikan kadar tekanan haba ayam pedaging, profil darah and organ limfoid. Dua puluh empat ayam pedaging jenis Cobb pada umur 21 hari dibahagikan kepada empat kumpulan rawatan. Tiga kumpulan rawatan diberikan tekanan haba iaitu T1 (1 ml/ kg berat badan dengan minyak soya), T2 (1 ml/ kg berat badan dengan asid askorbik) dan T3 (1 ml/ kg berat badan dengan ekstrak Cyperus rotundus dan minyak soya) serta T0 (1 ml/ kg berat badan dengan minyak sahaja) berperanan sebagak kumpulan kawalan. Ayam pedaging (T0) menunjukkan tindakbalas ketara ($p<0.05$) terhadap kenaikkan berat badan (BWG) dan pengambilan makanan (FI) serta pengurangan ketara ($p<0.05$) terhadap nisbah penukaran makanan (FCR) pada minggu kedua selepas administrasi ekstrak berbanding dengan kumpulan rawatan yang lain. Oleh yang demikian, kajian ini menunjukkan bahawa administrasi ekstak Cyperus rotundus tidak menunjukkan kesan terhadap tekanan haba singkat. Kesan positif hanya mampu dilihat terhadap tekanan haba kronik.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF SYMBOLS. UNITS AND ABBREVIATIONS

^0C	Degree of celsius
%	Percentage
ANOVA	Analysis of Variance
mm	Millimeter
cm	Centimeter
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
BW	Body Weight
ml	Milliliter
µg	Microgram
g	gram
kg	Kilogram
nmol	nanomoles
VC	Vitamin C
H_0	Null hypothesis
H_A	Alternative hypothesis
T0	Treatment 0
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
R1	Replication 1
R2	Replication 2
R3	Replication 3
MIC	Minimum inhibitory concentration

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

Hot weather is concerned on poultry in tropical countries with temperature above 30^{0}C (Moreki, 2008). It promotes heat stress in a sudden and unexpected event. Poultry are in fact unused to high ambient temperatures as they do not have sweat glands (Marandure, 2007), suggesting that birds are intolerance to heat under hot climatic conditions. This is contributed by the absence of sweat glands, numerous feathers and the vascularity only on the head and appendages, suppresses both the sensible and evaporative heat loss which discovered by Smith (taken by Marandure, 2007).

Birds are heat intolerant than cold, mortality is more likely expected due to heat stress rather than cold stress (Marandure, 2007). The fundamental problem is to permit normal functioning of the chemical processes by maintaining their body condition. Discovery made by Smith (taken by Marandure, 2007), broilers are food converters rather than food producers that produced quite amount of energy lost in the conversion process. This is further exaggerated with prolonged exposure to excess heat stress in sheds leading to considerable deaths. In fact, numbers of death occurred during hot weather due to inadequate ventilation. In a study by Alfataftah (1987), mortality rate of market age broilers is over 40% during a heat wave of August, year of 1985 in Jordan University Farm reaching the highest environmental temperature at 45.8^{0}C that went on for 3 days. Mortality rate results in economic loss (St-Pierre *et al.*, 2003). Therefore, ways to cope up this environmental effect should be assessed.

Heat stress can be neutralized by complex antioxidant systems that organism can develops. The antioxidant system can be served by supplementing antioxidants in diet.

Although synthetic antioxidants have been often used to protect against free radicals from heat stress, recent health concerns attracted much attention to the use of natural anti-oxidative compounds (Lu and Foo, 2000). Much interest has been drawn to plants and herbs for their medicinal and biological activities for the occurring compounds (Bhatia *et al.*, 2001). Flavonoids, one of the components of polyphenols are abundance in plants and present quite amounts in vegetables, spices, fruits, beverages and medicinal herbs. Middleton and Kandaswami (1994) claimed that flavonoids exhibit antimicrobial, antiplatelet, antitoxic and antiviral activities. Flavonoids also contain antigenotoxic as well as antioxidative activities (Bhouri *et al.*, 2010). Oxidative damage resultants from heat stress may be minimized by antioxidant defense mechanisms. Since the control of high environmental temperature by the cooling of animal buildings is impractical and high cost (Konca *et al.*, 2009), dietary manipulations serves as more beneficial and economical ways to alleviate the negative effects of heat stress.

Antioxidant measures to alleviate heat stress could be expressed via utilization of *Cyperus rotundus* as the natural anti-oxidative compound. According to Kilani-Jaziri *et al.* (2011), *C. rotundus* extracts potentially possess antimicrobial, antioxidant and antigenotoxic activities which could be derived from phenols and flavonoids. This make *C. rotundus* as a potential dietary supplement to cope up heat stress in broiler. *C. rotundus* is a sedge of the family of *Cyperaceae*, order of *Cyperales*. Some common names are nut grass and purple nutsedge. *C. rotundus* is colonial, herbaceous and perennial with fibrous roots that typically grows from 7 to 40 cm tall as well as reproduces extensively by rhizomes and tubers. It is known as the "world's worst" weed, a species known from more countries (at least 92) than any other weed that infest at least 52 kinds of crops worldwide (Holm *et al.*, 1997). It is able to grow in any kind of soils and survive under the highest temperatures known in agriculture. In China, *C. rotundus* has been used in traditional medicine. Extraction and isolation of compounds from it serves as medicinal properties such as reduction of fever, pain and inflammation (Urbasch, 2006). There is still no study made on broiler supplemented with *C. rotundus* as extract. Therefore, there is a scope to investigate the effect of feeding *C. rotundus* extract on the recovery problems pose by heat stress in broiler.

1.2 Justification

Heat stress on broiler leads to loss or reduce of value in terms of economic factor. Maintaining an optimum environment for broiler production involved great production cost (Marandure, 2007). As there will be higher used of electrical energy to control the ambient temperature which is impractical and costly (Konca *et al.*, 2009). Therefore, one of the practical way is via herb extracts supplementation. This is a process of supplementing plant with anti-heat stress properties to broiler. With no cost to obtain and grows actively in favorable condition, *Cyperus rotundus* could be one of the solution to suppressed the deleterious effects of heat stress. While exploring the potentiality of *C. rotundus* as a novel anti oxidative compound, this study will also serves as economic aspect to cut short cost on combating heat stress. This study will also be a leading ahead to anticipate the expected rise of global surface average of 2 to 3^{0}C during the next 50 years stated by Barry and Chorley (2003).

1.3 Objective

1. To study the effect of feeding *Cyperus rotundus* extract on feed intake and growth performance of heat stressed broiler
2. To study the effect of feeding *Cyperus rotundus* extract on common blood profile and lymphoid organs status of heat stressed broiler

1.4 Hypothesis

H_o: There is no effect of feeding *Cyperus rotundus* on any of the growth performance, blood profile and lymphoid organs parameters of heat stressed broiler

H_A: There is difference in at least one parameters of the growth performance, blood profile and lymphoid organ of *Cyperus rotundus* fed broiler and that of the control group.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Cyperus rotundus is commonly known as nut grass or purple nutsedge grass. It belongs to the *Cyperacea* family and grows in tropical, subtropical and temperate regions. It is known as one of the earliest and important edible herbs as well as having wide spectrum activity in biological systems (Kilani-Jaziri *et al.*, 2009). Yang (1997) added that *C. rotundus* is still used as a traditional medicine to improve blood circulation especially in gynecological diseases due to blood stagnation.

2.1.1 Botanical Characteristics of *Cyperus rotundus*

The description of plant below is referred from the United States of Department Agriculture, Natural Resources Conservation Service, USDA NRCS, (n.d.). *Cyperus rotundus* is a perennial, colonial and herbaceous with fibrous roots. It can grow up to 7 to 40 cm tall and reproduces by tubers and rhizomes. Rhizomes in the soil grow in any direction be it upward or downward. Those growing upward, reaching the soil surface will enlarge forming a 2 to 25 mm of basal bulb diameter, producing roots, shoots and other rhizomes. While those growing downward or horizontally become chains of tubers or individual tubers, about 12 mm thick and range from 10 to 35 mm long at maturity. Other characteristics are stems of 10 to 50 cm tall and of triangular cross section as well as having bisexual flowers, each with three stamens and three stigmas on a pistil. It is a C_4 plant that could assimilate CO_2 at higher light intensities and temperature compare to C_3 pathway plant. C_4 plant grow best at temperatures of tropical and subtropical regions.

Pictures 2.1 Whole plant of *Cyperus rotundus*
Source: *http://dpipwe.tas.gov.au/Documents/Cyperus-rotundus-assessment.pdf*

2.1.2 Weed Characteristics of *Cyperus rotundus*

Cyperus rotundus is known as the "world's worst" weed. This entitled is due to the difficulty to control it once established and also the ineffectiveness of herbicides. Following establishment, agriculture and natural ecosystems are greatly affected by the displacing native plants or by the changes on food or shelters for native animals.

This rapid growing plant form colonies really fast through their ability to produce an extensive systems of tubers and rhizomes. In fact, documented studies showed the reduction yields of cotton, rice, corn, sugar cane, vegetables and other crops. In extreme cases, both sugarcane and sugar yields were reduced by 75% and 65%. Similarly, cornfields yield in Colombian was reduced by 10% if *C. rotundus* was allowed to grow for 10 days, reduction to 30% is noted if allowed for 30 days. Effects were as well demonstrated by Holm *et al.*, (1977) in other crops. This resource competition was even noted in humid regions discovered by Rochecouste used by USDA NRCS (n.d), in which the production of *C. rotundus* shoots and tubers restrict water availability to sugarcane. It is estimated by Holm *et al.* (1977) that 815 kilograms of ammonium sulfate, 320 kilograms of potash and 200 kilograms of phosphate per hectare are possibly stored in *C. rotundus*.

2.1.3 Benefits of *Cyperus rotundus*

There are several uses of *Cyperus rotundus*. In China, it is used as traditional medicine and in landscaping as well as soil binder in India. However, it does not suite to be used as fodder since it becomes fibrous with age quickly, but it can serve that purpose if desirable plants are deficit (Holm *et al.*, 1997). Wills (1987) showed that tuber extracts of *C. rotundus* may act as a muscle relaxant and reduce nausea. To add more, the extractions and isolation of compounds pose some medicinal properties such as the reduction of pain, fever and inflammation. Study made by Kilani-Jaziri *et al.* (2011), *C. rotundus* extract contained antimicrobial, antioxidant and antigenotoxic activities against superoxide radical. These potent phytochemicals were derived from compounds such as flavonoids and phenols.

2.1.4 Extraction of *Cyperus rotundus*

Extraction methods use to investigate phenolic contents of *Cyperus rotundus* (Kilani-Jaziri *et al.*, 2011) are aqueous, ethyl acetate, methanol and total oligomer flavonoids (TOF) extract. Aqueous extract utilizes partially or whole plant. It is reduces to coarse powder after it is dried at room temperature. Follow by extraction of powdered plants by boiling water for 15 to 20 min. The resultant crude extract is then filtered and lyophilize (aqueous extract). While ethyl acetate extract and methanol extract are obtained by soxhlet extraction for 6 hours. These types of extract use different polarities which are concentrated to dryness. Resultant residue is kept at 4^0C. Both extracts are again suspended in DMSO. In TOF extraction, powder is macerated in water/acetone mixture (1v/2v) for a day with continuous stirring. This is to obtain enriched TOF extract. The extract is then filtered and under low pressure, the acetone is evaporated as to get aqueous phase. The aqueous phase contained some tannins which are precipitated with an excess of NaCl at 5^0C for 24 hours, filtered and recovered. It is concentrated and precipitated with an excess chloroform after it has been extracted with ethyl acetate. The precipitated is then separated yielding TOF extract that dissolves in water using Kilani-Jaziri *et al.* (2011) protocols.

2.2 Phytochemical Study and Metabolite Content

Study by Kilani-Jaziri *et al.*, (2011) found out that *Cyperus rotundus* gave various amounts of flavonoids, coumarins and tannins in the aqueous, methanol and Total Oligomer Flavonoids (TOF) enriched extracts. On a side note, his study showed that ethyl acetate extract gave out sterols and flavonoids. The metabolite contents of these extracts tested are presented in Table 2.1.

Table 2.1 Quantitative phytochemical Screening (%) of extracts from *Cyperus rotundus.*

Metabolites	Extracts			
	Aqueous extract	TOF extract	Ethyl acetate extract	Methanol extract
Polyphenols (Gallic acid equivalent)	260 ± 11	670 ± 20	440 ± 12	330 ± 11
Flavonoids (quercetin equivalent)	200 ± 14.5	340 ± 23	320 ± 15	290 ± 13
Tannins (mg/ 100g)	59. 61 ± 8.5	229 ± 13.25	117.1 ± 8	68.7 ± 6.25
Sterols (%)	-	-	2.75 ± 0.25	-

\- Undetectable
Source: Kilani-Jaziri *et al.* (2011)

TOF enriched extract have a considerable amount of flavonoids and total phenolic compounds compare to the other three extracts. In details, TOF enriched extract of 1 mg was similar to Gallic acid of 670 µg and quercetin of 340 µg. While 1 mg of aqueous, ethyl acetate and methanol extracts were equivalent to 260, 330 and 440 µg of Gallic acid and 200, 290 and 320 of quercetin µg. However, Kilani-Jaziri *et al.*, (2011) used a past study and stated that TOF enriched extract came with the highest tannins content of 229 mg/100g and followed by ethyl acetate extract with 117.1 mg/ 100g. The other two extracts recorded less tannin contents with 68.7 and 59. 61 mg/100 g in methanol and aqueous extracts respectively. Sterols content was noted only in ethyl acetate extract.

2.2.1 Antimicrobial Activity of *Cyperus rotundus*

Cyperus rotundus poses some levels of antibacterial effect against bacterial strains. These tested strains are *Salmonella typhimurium, Staphylococcus aureus, Salmonella enteritidis, Enterococcus faecalis and Escherichia coli.* The antibacterial activity of *C. rotundus* on Gram-positive and Gram-negative were tested by Kilani-Jaziri *et al.* (2011) giving value of minimum inhibitory concentration (MIC) 0.25 mg/ml to over 5 mg/ml and value of minimal bacterial concentration (MBC) from 0.5 mg/ml to over 5 mg/ml. From his study, *C. rotundus* showed different activity on each extracts used. Interestingly, only two extracts, TOF enriched extract and ethyl acetate, showed antibacterial effect while aqueous and methanol extracts showed no inhibitory effect. This was due to the high amounts of flavonoids and other phenolic compounds in TOF and ethyl acetate extract. These phenols and flavonoids gave rise to antimicrobials activity (Rodriguez Vaquero *et al.*, 2007). The antimicrobial activity of *C. rotundus* is presented in Table 2.2.

Table 2.2 Antimicrobial activity of *Cyperus rotundus* extracts, expressed as MIC[a].

Extracts	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Salmonella anteritidis*	*Enterococcus faecalis*
TOF extract	0.5	>5	0.5	1	0.25
Aqueous extract	>5	>5	>5	>5	>5
Ethyl acetate extract	2.5	5	2.5	2.5	2.5
Methanol extract	5	>5	>5	>5	>5
Ampicillin[b]	0.0015	0.006	0.0039	0.0019	0.0025

[a] Values in mg/ml, means of three requirements.
[b] Positive control
Source: Kilani-Jaziri *et al.* (2011)

Cyperus rotundus also exhibits active compounds like tannin. Haslam (1996) demonstrated that tannins are active compound of several medicinal plants having antibacterial activity. In fact, Hagerman and Butler (1981) showed that tannins can form irreversible complexes with proline-rich proteins, inhibiting cell wall protein synthesis. The ability of forming complexes with macromolecules and interfering biological systems could explained its combination with a polyphenolic nature. Thus, this explain the high tannin content in TOF extract which is high in phenolic compounds. This property alone could explained the antibacterial mechanisms of plant extracts. Similarly, De-Bruyne *et al.* (1999) reported that several in vitro assays showed potent interactions of tannins with biological systems such as bacteria, molluscs, viruses as well as enzyme inhibiting, antioxidant and radical scavenging properties.

2.2.2 Superoxide Radical-Scavenging Activity

The antioxidant properties of *Cyperus rotundus* are at different levels for different extract used. Indeed, it posed considerable antioxidant activities, but not significantly in all extracts. Although aqueous extract has quite similar amount of flavonoids as the methanol extract, they showed inferior effect on antioxidant characteristics (Kilani-Jaziri *et al.*, 2011). Thus, suggesting that TOF extracts and ethyl acetate extracts gave better effect on the antioxidant activity. The antioxidant order for TOF and ethyl acetate tested extracts followed their respective polyphenol and flavonoid content. Bors *et al.* (2001), claimed that polyphenols are the most active antioxidant derivatives in plants, although other studies by Meda *et al.* (2005) had stated that the phenolic content of several plant extracts did not correlate with the antioxidant activity.

Table 2.3 Superoxide anion (O_2) radical-scavenging activity of extracts from aerial parts of *Cyperus rotundus*[a].

Extracts	Dose µg/assay	% Inhibition	CI_{50} (µg/ml)
Ethyl acetate	1000	74.34 ± 4.6	50
	300	66.7 ± 3	
	100	56.7 ± 2.8	
	30	47 ± 0.3	
	10	0.7 ± 1.05	
TOF extract	1000	72.1 ± 2	60
	300	64.2 ± 6	
	100	47 ± 2.4	
	30	42.8 ± 9	
	10	35.8 ± 2.4	
methanol extract	1000	66.5 ± 3.2	90
	300	50.8 ± 2.2	
	100	50.52 ± 2.1	
	30	38.5 ± 1.5	
	10	12.1 ± 7.5	
Aqueous extract	1000	53 ± 7.2	370
	300	47 ± 0.9	
	100	29.9 ± 7.2	
	30	25.7 ± 1.5	
	10	5 ± 3	
Quercetin[b]	1000	64.96 ± 2	360
	300	34.5 ± 3	
	100	20.1 ± 1.6	
	30	9.17 ± 1.3	
	10	1.38 ± 2.2	

[a] Values in mg/ml, means of three requirements.
[b] Positive control
Source: Kilani-Jaziri *et al.* (2011)

2.2.3 Proliferation of L121O Leukemia Cells from Tuber Extract

The relationship between extracts concentration and their cytotoxic effect was investigate by MMT assay on L1210 cells (Kilani *et al*, 2008). MMT is a yellow watersoluble tetrazolium salt which acts as indicator. MMT is converted to dark blue formazan by metabolic active cells through the reduction of tetrazolium ring's cleavage. The study gives a profound cytotoxic effect. TOF enriched extract at 50-800 µg/ml reduces proliferation of L1210 cells by 0.61 (61%).

On the other hand, ethyl acetate extract showed the strongest cytotoxic effect on the cells with 78.92% proliferation. Again, the strong cytotoxic effect of both extracts from *Cyperus rotundus* tubers were addressed to the components of coumarins, flavonoids and total polyphenols of those extracts. In fact, Musonda and Chipman (1998) showed that flavonoids are capable of modulating cell signal in cell functions. Thus, altering cytotoxic and proliferation in cancer cell lines (Kuntz *et al.*, 1999). This was further supported by Hirano *et al.* (1994). His studies showed that flavonoids posed cytotoxic effects on human cell lines- leukemia cells and also (Benavente-Garcia *et al.*, 1997) on ovarian cancer cells.

Previous studies by Harbone (1980) reported that *Cyperus rotundus* extracts poses luteolin. Leuteolin is a polyphenolic compounds found in foods of plant origin and subclass of flavonoiods. Lee *et al.* (2002) further elaborated that luteolin showed antioxidant and antimutagenic effects and in actively on various tumours such as pancreatic tumour cells and leukemia cell line. Minor components of these extracts are actually able to give cytotoxic effect (Yu *et al.,* 2004).

2.3 Heat Stress in Poultry

The expression of heat stress in poultry production can be narrowed down into chronic and acute. According to discovery by Emery (taken by Al-Fataftah and Abu-Dieyeh, 2007), acute stress is short and sudden periods of really high temperature, whereas chronic heat stress is the period extending of elevated temperature. Reduction of feed intake, growth rate, feed efficiency and carcass quality as well as health are the effects brought by chronic heat stress on broiler reared in open-sided poultry houses. Howlider and Rose (1989) further added that extend periods of elevated temperature lengthen the time to reach market weight and promote mortality.

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