UNIVERSITI PUTRA MALAYSIA

RESPONSE OF BROILER CHICKENS TO DIETARY SUPPLEMENTATION OF L-GLUTAMINE AND L-GLUTAMATE UNDER STRESSFUL CONDITIONS

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By

MAJID SHAKERI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

I dedicate this thesis to my beloved parents and my brothers for their unconditional love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: **Professor Zulkifli Idrus, PhD**

Institute: **Institute of Tropical Agriculture**

High stocking density (HSD) and delay in placement are practices that may impair growth performance and well-being of broiler chickens. Dietary supplementation with L- glutamine and L-glutamate (LG) has been suggested to improve poultry performance under stressful conditions. Thus, this research was conducted to evaluate the effect of LG supplementation on performance, gut morphology, physiology and well-being of broiler chickens subjected to different stocking densities and posthatch holding times. In Chapter 3, one-day-old broiler chicks were stocked either at 10 birds/m^2 or 15 birds/m^2 and assigned to four dietary treatments namely (i) basal diet (ii) basal diet supplemented with 0.5% LG from 1 -21 days of age (LG3), (iii) basal diet supplemented with 0.5% LG for from 1 – 42 days of age (LG6), (iv) and basal diet supplemented with virginiamycin from 1 – 42 days of age (VM). The results indicated that neither LG3 nor LG6 had beneficial effects on growth performance, duodenal villi and crypt depth, gut *E. coli* and *Lactobacilli counts*, and incidence of food pad dermatitis. VM improved growth performance, villi length and decreased the population of *E. coli.* Irrespective of LG and VM supplementation, the high stocking density impaired growth performance, and villi length, and increased incidence of foot pad dermatitis. The high stocking density elevated serum levels of ceruloplasmin (CP) , α1 acid glycoprotein (AGP), corticosterone (CORT), and heterophil / lymphocyte ratios (HLR) as compared to the low stocking density. In Chapter 4, broiler chicks were subjected to either immediate placement or delayed placement (24 hours after arrival). Chicks from each placement time were supplemented with LG at 1% from 1 -21 day of age or unsupplemented diet. Chicks subjected to 24 hours of delayed placement had larger yolk sac, and poorer early weight gain (during 1 -21 days of age) than their control counterparts. However, the final weight gain and feed conversion ratios (FCR) of the chicks subjected to immediate and delayed placement were not significantly different. Delayed placement had no significant effect on gut morphology, CP, AGP, OVT, and duodenal heat shock protein (HSP) 70 density at 21 days of age.

Supplementing birds with LG increased duodenal villi length and crypt depth at 21 days of age, and improved final weight gain, FCR and survivability when compared to controls. The CP, AGP, OVT and HSP 70 density of chicks subjected to delayed placement were elevated when compared to controls. The present findings suggested that LG supplementation at 1% from day 1 – 21 days of age can improve weight gain, FCR, and gut morphology of chicks subjected to 24 hours of delayed placement. Supplementing chicks with LG may also aid chickens to cope with stresses through elevation in APP and HSP 70.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysiaebagai memenuhi keperluan untuk ijazah Master Sains

RESPONS AYAM PEDAGING KEPADA PEMAKANAN TAMBAHAN L- GLUTAMINA DAN L- GLUTAMAT DALAM KEADAAN TERTEKAN

Oleh

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Jun 2014

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Tahap kepadatan tinggi (HSD) dan kelewatan dalam penempatan anak ayam adalah dua faktor penting yang boleh menyebabkan tindak balas tekanan fisiologi dan menjejaskan prestasi pertumbuhan dan fungsi imun bagi ayam . Baru-baru ini , L- glutamina dan L- glutamat (LG) telah dicadangkan untuk meningkatkan prestasi ayam daging di bawah keadaan tekanan. Oleh itu, kajian ini dijalankan untuk menilai dua tahap pemakanan LG iaitu 0.5% dan 1% ke atas prestasi pembesaran , morfologi usus, pengeluaran antibodi, duodenum , heat shock protein (HSP) 70 penyataan, dan parameter darah tahap serum protein fasa kritikal (ovotransferrin , ceruloplasmin dan asid Glycoprotein alfa 1), kepekatan plasma corticosterone dan nisbah heterophil / limfosit) dalam ayam daging tertakluk kepada kepadatan bekalan dan penempatan tertangguh selama satu hari umur. Dalam percubaan pertama , anak ayam berumur satu hari diberikan kepada 32 kurungan (floor pen) (1.7m × 1.4m) sebanyak 10 anak ayam/m^2 dan 15 anak ayam/ m^2 dan diberikan kepada empat rawatan pemakanan iaitu (i) suplemen diet asas (ii) suplemen diet asas dibekalkan sebanyak 0.5 % LG pada tiga minggu pertama , (iii) suplemen diet asas ditambah sebanyak 0.5% LG selama 6 minggu, (iv) dan suplemen diet asas ditambah sebanyak 0.02 % virginiamycin selama 6 minggu. Pengambilan makanan , nisbah pertukaran makanan (FCR) dan berat badan badan direkodkan setiap minggu. Sampel darah dan duodenum diambil pada hari ke-42. Keputusan eksperimen pertama menunjukkan bahawa pemberian suplemen LG sebanyak 0.5% selama 3 minggu pertama dan 6 minggu berikutnya tidak menjejaskan prestasi pertumbuhan, FCR, parameter darah dan tempoh penyerapan ($p > 0.05$) berbanding dengan suplemen diet asas dalam kepadatan stok yang sama. Walau bagaimanapun, dengan memasukkan Virginiamycin didapati signifikan dengan bertambah baiknya parameter tersebut ($p < 0.05$). Ketumpatan stok tinggi menjejaskan berat badan, FCR , tempoh penyerapan , tindak balas antibodi dan peningkatan paras fasa kritikal protein serum, nisbah

heterophil / limfosit dan plasma corticostrone ($p < 0.05$) berbanding dengan kepadatan stok rendah. Dalam kajian yang kedua , anak ayam telah diberikan kepada 20 kurungan (2.5m × 1.4m) sebanyak 25 burung /kurungan selama 42 hari dan dibekalkan dengan diet asas atau suplemen diet asas dengan 1% LG untuk tiga minggu pertama. Anak ayam umur sehari daripada setiap kumpulan diet yang berlainan tertakluk kepada penempatan serta-merta atau penempatan yang bertangguh (24 jam selepas ketibaan). Pengambilan makanan , FCR dan berat badan badan direkodkan pada setiap minggu. Sampel darah dan duodenum diambil pada hari yang ke-21. Gabungan 1% LG mempunyai kesan positif ke atas tempoh serapan , tahap fasa kritikal serum protein dan HSP 70 ($p < 0.05$) berbanding dengan kumpulan yang menerima diet asas selama 21 hari . Begitu juga, FCR dan nilai berat badan sebanyak 1% LG mempunyai signifikan yang jauh lebih baik daripada yang diberi pemakanan diet *asas* pada akhir eksperimen. penempatan tertangguh anak ayam tidak mempunyai kesan yang ketara ke atas prestasi pertumbuhan , morfologi usus dan parameter darah di kedua-dua kumpulan pemakanan. Oleh yang demikian, hasil kajian ini menunjukkan bahawa suplemen 0.5% LG dalam diet tidak berfungsi di bawah tekanan kepadatan stok tinggi dan 1% LG meningkatkan kesihatan ayam daging dan meningkatkan prestasi di bawah tekanan penempatan tertangguh.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

LG	Mixture of L- glutamine and L-glutamate
AGP	Acid glycoprotein
APP	Acute phase protein
CP	Ceruloplasmin
CORT	Corticostrone
FCR	Feed conversion ratio
FPD	Foot pad dermatitis
g	Gram
GIT	Gastrointestinal tract
GLN	Glutamine
GLU	Glutamate
h	Hour
HLR	Heterophil to Lamphocyte
HSD	High Stocking Density
HSP	Heat shock protein
LSD	Low stocking density
mg	Mili gram
ml	Mili litter
nm	Nano meter
OVT	Ovotransferin
VM	Virginiamycin
µl	Micro litter

CHAPTER 1

GENERAL INTRODUCTION

Over the past several decades the poultry meat industry has been growing rapidly, particularly in developing countries (Taha, 2003). In 2008, approximately 71.4 million metric tons of poultry meat was produced and this was 5 % more compared to 2007productions (US Department of Agriculture, 2009). With the estimation of 2.6% annual growth, poultry meat production will reach 67 million metric tons by 2013 globally. The current strategy of the industry is to increase production efficiency through reducing the growing time period. Accordingly, researchers are constantly looking for alternatives and solutions to achieve this target. One of the areas among the factors and conditions that are of increasing interest to researchers and industry is animal welfare. Animal welfare is directly related to the housing system (Hubrecht and Kirkwood, 2010), nutrition and environment conditions such as stocking density (Broom, 1993).

Stocking density is defined as the number of chicks per specific amount of area per bird. High stocking density can reduce animal welfare by decreasing the quality of the environment and increasing competition for access to water and feed (Knowles *et al.*, 2008). Optimum stocking density for broiler chicken is 10 birds per square meter (Thomas *et al.*, 2004). Generally, high stocking density (HSD) may negatively affect performance, liveability, litterer moisture, feed efficiency (Sanotra *et al,*. 2001; Dozier *et al.*, 2005; Estevez, 2007; Skrbic and Lukic, 2009) and decrease the amount of production due to economic losses (Braun *et al.*, 2010). Moreover, it increases microbial activity and promotes higher incidences of dermatitis among chickens through increase moisture of litterer (Bessei, 2006). However, from the economic viewpoints of the market and producers, apart from the negative effects of high stocking density on the welfare of chickens (Hall, 2001), produce more meat is more considered than the weight of each chicks (Bilgili and Hess, 1995), which cause higher profitability (Shanawany, 1988).

The handling of chicken after hatching is a crucial matter which is directly connected with optimum growth performance and good welfare. Under commercial procedures, chicks are subjected to various processes such as sorting, vaccination, preparation and transportation which can physiologically affect them. Transportation of animals to the farm has been considered as an important factor which can be related to food quality and animal welfare (Keeling, 2005; Marahrens *et al.*, 2011) and also cause delay placement which is a negative factor in chickens performance. Delayed placement has been shown to cause dehydration and depletion of yolk sac reserves (Vieira *et al.* 2005). Earlier studies have shown a relation between delayed placement and depression in growth performance of chickens (Mahmoud and Edens, 2012; Peng and Guo, 2010). In fact, delivery to farms has been known to occur delays in chicken placement for as long as 24-48 h.

As a result, it is common in the poultry industry to supplement chickens with antibiotics to eliminate the negative effects of high stocking density on growth

performance and gut health. However, the concern on antibiotic resistance - particularly in human – has led the poultry industry and researchers to look for the consumer-farmer friendly alternatives. Among the several alternatives to fortify the diets with dietary nutrition, glutamine supplementation is considered as an important factor in promoting the well-being and increasing the health of chickens. As shown by Andrews and Griffiths, (2002), Yi *et al.* (2001) and Aledo, (2004) glutamine supplementation can increase villa length and act as an energy source for the intestinal tissue and immune cells. During stress incidence, the damage to the intestinal epithelium may increase, and therefore, it may enhance the pathogens chances to enter into the body (Adjei *et al,.* 1994). Glutamine may function to decrease the incidence of infection by improving the gut health and epithelial permeability (Andrews and Griffiths, 2002; Medina, 2001).

Thus, the main objective of the this study was to investigate the effects of L- glutamine and L-glutamate (LG) supplementation on factors affecting performance of broiler chickens which were subjected to stressful conditions.

Therefore, the objectives of this study were:

(1) To investigate the effects of L- glutamine and L-glutamate and antibiotic dietary supplements on growth performance, intestinal morphology and microflora, and blood parameters in broiler chickens stocked at different densities under the hot tropical environment.

(2) To evaluate the effects of dietary L- glutamine and L-glutamate supplementation, and delayed placement on the growth performance, intestinal morphologyand physiological stress responses in broiler chickens under the hot tropical environment.

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Majid Shakeri was born in Mashhad, Iran, on 21 March 1985. He received his Bachelor of Science degree (Animal science) in University Iran, Birjand from 2006 to 2008. In 2011, he pursued his Master of Science degree majoring in Poultry Reproduction at the Institute of Tropical Agriculture, Universiti Putra Malaysia. He had participated in many research projects during his master degree. He is currently working with some companies related to his subject in Iran.

LIST OF PUBLICATIONS

Journal

1. M. Shakeri, I. Zulkifli, A. F. Soleimani, P.D. Eckersall, A. Anna Aryani, S. Kumari, and Faez Firdaus Jesse Abdullah. (2014). Response to dietary supplementation of L-glutamine and L-glutamate in broiler chickens raised at different stocking densities under the hot, humid tropical conditions. (Accepted by Poultry Science Journal).