UNIVERSITI PUTRA MALAYSIA

EFFECT OF INSECT CHITIN AS FEED SUPPLEMENT IN SELECTED CLINICAL BIOCHEMISTRY AND GROWTH PERFORMANCE OF THE BROILER CHICKENS

SITI NUR AFIQAH BINTI JUAHARI

EFFECT OF INSECT CHITIN AS FEED SUPPLEMENT IN SELECTED CLINICAL BIOCHEMISTRY AND GROWTH PERFORMANCE OF THE BROILER CHICKENS.

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Effect Of Insect Chitin As Feed Supplement In Selected Clinical Biochemistry And Growth Performance Of The Broiler Chickens" by Siti Nur Afiqah binti Juahari and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 - Project.

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DEDICATIONS

This thesis is dedicated to

My parents,

Juahari bin Seran & Partini binti Parmon

For all their support, unconditional love and prayers.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

KESAN SERANGGA CHITIN

SEBAGAI MAKANAN TAMBAHAN KEPADA PARAMETER BIOKIMIA KLINIKAL TERTENTU DAN PRESTASI PERTUMBUHAN DALAM AYAM PEDAGING.

Oleh

SITI NUR AFIQAH BINTI JUAHARI

2015

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Abstrak

Sebanyak 36 ekor anak ayam pedaging baka Ross berumur 21 hari dibahagi secara rawak kepada 1 daripada 3 pelakuan dalam kajian yang dijalankan untuk menentukan kesan makanan tambahan daripada chitin serangga (IC) terhadap pertumbuhan dan komposisi darah. Diet ujikaji terdiri daripada satu diet kawalan berasaskan jagung, mil kacang soya, bijirin dan bahan sampingan bijirin, pelakuan

kedua terdiri daripada diet kawalan ditambah dengan 1 g IC / kg diet, dan pelakuan ketiga terdiri daripada diet kawalan ditambah dengan 2 g IC / kg diet. Setiap pelakuan mempunyai 3 replikat dengan setiap replikat mengandungi 4 ekor ayam. Prestasi ayam pedaging dan komposisi metabolit darah diukur pada permulaan dan setiap 7 hari dalam tempoh 21 hari ujikaji. Sepanjang tempoh 21 hari ujikaji, ayam dalam kumpulan kawalan telah mencatatkan purata harian (ADG) yang tertinggi apabila di bandingkan dengan pelakuan yang mengandungi IC sebagai makanan tambahan tetapi perbezaan adalah tidak ketara. Tidak terdapat perbezaan yang ketara bagi ADG ayam diberi makan 2 g IC daripada ayam diberi makan 1 g IC walaupun ADG ayam bagi pelakuan 2 g IC adalah lebih baik.. Profil metabolit darah untuk semua diet pelakuan adalah sama. Kesimpulannya, makanan tambahan IC tidak memberi keasn terhadap peningkatkan ADG dan metabolit darah ayam pedaging. Kekurangan tindak balas ayam pedaging terhadap makanan tambahan IC dalam kajian ini berkemungkin disebabkan mutu IC yang rendah atau tahap IC diuji dalam kajian ini adalah terlalu rendah untuk haiwan untuk bertindak balas.

Kata kunci: *chitin serangga (IC), prestasi pertumbuhan, hematologi, serum biokimia, ayam pedaging.*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

EFFECT OF INSECT CHITIN

AS FEED SUPPLEMENT

IN SELECTED CLINICAL BIOCHEMISTRY AND

GROWTH PERFORMANCE OF THE BROILER CHICKENS.

by

SITI NUR AFIQAH BINTI JUAHARI

2015

Supervisor: Dr. Yusof Hamali Ahmad

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Abstract

A total of 36 twenty one day Ross broiler chicks were randomly allocated to 1 of 3 treatments in a study conducted to determine the effects of dietary supplementation of insect chitin (IC) on growth and blood composition. The experimental diets consisted of an unsupplemented basal control diet based on corn, soybean meal, and grain by-products, second treatment consisted of basal diet supplemented with 1 g

IC/kg of diet, and third treatment consisted of 2 g IC/kg of diet. Each treatment was fed to 3 replicate pens of birds, with 4 birds per pen. Broiler performance and blood metabolite indices were measured at the beginning and every 7 day during the 21 day experimental period. Throughout the 21 day experimental period the control group broilers recorded the highest average daily gain (ADG) than the other treatments but the difference was not significant. There was no significant difference in ADG of broilers fed 2 g of IC than broilers fed 1 g of IC even though broilers supplemented with 2 g IC recorded higher ADG. The blood metabolite profiles for all treatment diets were similar. In conclusion, dietary supplementation of IC appeared not to improve ADG and blood metabolites of broilers. Poor responses of broilers to IC supplementation in this study was probably due to low quality IC product or the level of IC tested in this study was too low for the animal to respond.

Keywords: *insect chitin (IC), growth performance, haematological, serum biochemistry, broiler chickens.*

Chapter 1

1.1 Introduction

Chitin is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked with β-(1-4) glycosidic bond, where N-acetyl-D-glucosamine units are predominant in the polymeric chain. The deacetylated form of chitin refers to chitosan (Figure 1). Chitin and chitosan can be found as supporting materials in many aquatic organisms, terrestrial organisms, and some microorganisms (Tokura and Tamura, 2007). The interest in chitin study originates from the study of behaviour and chemical characteristics of lysozyme. This enzyme dissolves certain bacteria by cleaving the chitinous material of the cell walls. This made chitin resulted in a wide variety of medical applications that has been reported over the last three decades. Many reports showed that chitin has been implicated in a wide variety of applications including health beneficial and antimicrobial uses (Yalpani *et al.*, 1992; Howling *et al.*, 2001; Shahidi and Abuzaytoun, 2005). Antimicrobial effect brings benefit to the animal host in term of better gastro-intestinal activity and therefore promotes better growth and health status. Nowadays, commercially, chitins and chitosans are produced from biowastes obtained from aquatic organisms. The production of chitin and chitosan from biowastes of aquatic organisms in industrial scale appear in inconsistent physicochemical characteristics of products because of seasonal and variable supply of raw materials as well as variability and difficulties of process conditions (Nwe and Stevens, 2008). To overcome these problems, terrestrial organisms like insects, terrestrial crustaceans, and mushrooms are considered as alternative sources for the production of chitin and chitosan. Recently, the production of chitin and chitosan

from insect sources has drawn increased attention because chitin is also a primary component in insect cuticles. Therefore, insects are an alternative source of chitin and, consequently, of chitosan. First, insects possess enormous biodiversity and represent 95% of the animal kingdom. Therefore, they offer a tremendous potential as a natural resource for chitin and chitosan production. Until now, however, only limited numbers of insect species have been documented to be sources of chitin. One of the local insect that has the potential to be the source of chitin and chitosan is house cricket (HC) which is the most commonly found among cricket species in the country. The HC is easily adapted to domestic rearing and has not been seriously studied as a potential source of chitin or chitosan. In the present study, the prospect for using the exoskeleton of HC as a raw material for chitin will be tried out. This includes the extraction of chitin from HC by deproteinization and demineralization and chitosan by deacetylating the chitin. This study will evaluate chitin from HC comprehensively as alternative chitin source for poultry.

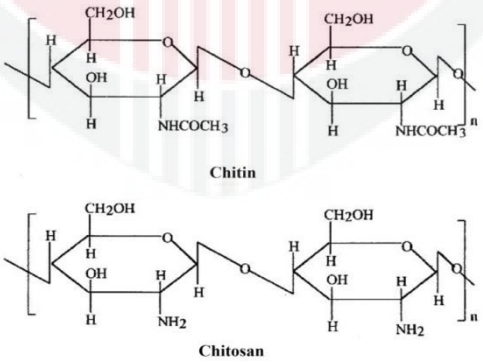

Figure 1 Chemical structure and the comparison between chitin and chitosan

1.2 Justification or rationale of study

The production of chitin and chitosan from biowastes of seafood by products in industrial scale which appear in inconsistent physicochemical characteristics variable supply of raw materials and processing (Nwe and Stevens 2008). To overcome these problems, terrestrial organisms like insects, terrestrial crustaceans, and mushrooms are considered as alternative sources for the production of chitin and chitosan. Chitin is a primary component in insect cuticles. Chitin is also found in internal structures of insects. Next to its occurrence in tracheal cuticles, it is also a constituent part of the peritrophic matrices that line the inner surface of the gut in many insects, protecting the intestinal epithelium from mechanical disruption, radical oxygen species and invasion by microorganisms (Barbehenn and Stannard, 2004). Furthermore, insect cuticles have lower levels of inorganic material compared to crustacean shells, which makes their demineralization treatment more convenient. Therefore, insects can be used as an alternative source of chitin and, consequently, of chitosan. Insects also possess enormous biodiversity and represent 95% of the animal kingdom.

1.3 Objective

To evaluate the growth performances and selected clinical biochemistry parameters in the broiler chickens.

1.4 Hypothesis

Supplementing broiler chickens diets with insect chitin will improve growth performances and selected clinical biochemistry parameters.

Chapter 2

Literature Review

2.1 Chitin structure, function and importance

Chitin is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked with β-(1-4) glycosidic bond, where N-acetyl-D-glucosamine units are predominant in the polymeric chain (Tokura and Tamura, 2007). Chitin structure is identical to cellulose except that chitin has acetamide groups (-$NHCOCH_3$) at the C-2 positions (Figure 2). While chitosan is a linear polymer of α (1-4)-linked 2-amino-2-deoxy-β-γ-glucopyranose and easily derived by *N*-deacetylation. Chitin is estimated to be produced annually almost as much as cellulose. Chitin naturally is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of the invertebrates. Chitin and chitosan are now produced commercially in India, Poland, Japan, the US, Norway, and Australia (Dutta, *et al.*, 2004).

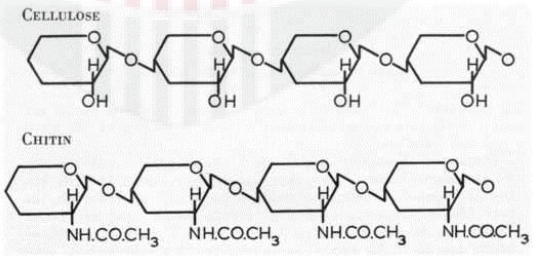

Figure 2 Comparison of the structure between chitin and cellulose

According to the same author, these naturally abundant and renewable polymers have excellent properties such as, biodegradability, bio-compatibility, non-

toxicity, and adsorption. Chitosan however is believed to be more versatile than cellulose due to the presence of NH_2 groups. On the other hand, few authors also reported on the biomedical applications of chitin and chitosan. It also functions as light but mechanically strong scaffold material and is always associated with cuticle proteins that mainly determine the mechanical properties of the cuticle (Merzendorfer and Zimoch, 2003). Andersen *et al.* (1995) do reported that the cuticle can be found in highly conserved in various insect orders, while some of them are restricted to specific body parts and others contain repeats of hydrophobic residues that seem to be linked with cuticle rigidity. Chitin also function as a permeability barrier between the food bolus and the midgut epithelium in insects that enhanced the digestive processes and protect the brush border from mechanical disruption as well as from attack by toxins and pathogens (Tellam, 1996; Merzendorfer and Zimoch, 2003).

2.2 Characteristic of chitin

Study done by Rudall and Kechington (1973), later followed by Kramer and Koga (1983) reported that under X-ray diffraction analysis, chitin is a polymorphic substance that occurs in three different crystalline modifications, termed α, β and γ chitin. They mainly differ in the degree of hydration, in the size of the unit cell and in the number of chitin chains per unit cell. The three chitin variants differ in their degree of hydration, in their size of the unit cell and in the number of chitin chains per unit cell. β-chitin is the most crystalline and compact form where the chains are arranged in an antiparallel fashion (Carlstrom, 1957). α- chitin consists of parallel chains, while in γ-chitin; two out of three chains are parallel with the third oriented

in the opposite direction. The distribution of the polymorphic forms is not related to taxonomy, as different forms may occur in one organism, providing different functional properties: α-chitin is by far the most abundant form, and is usually found where extreme hardness is required (Rudall and Kenchington, 1973). β- and γ-chitin seem to provide toughness, flexibility and motility and may have physiological functions other than support (Muzzarelli, 1988). The poor solubility of chitin is a result of the close packing of chains and its strong inter- and intramolecular bonds among the hydroxyl and acetamide groups (Urbanczyk *et al.*, 1997). The inability of α-chitin to swell upon soaking in water is explained by the extensive intermolecular hydrogen bonding. On the other hand, β- and γ-chitin lacks these inter chain hydrogen bonds, and therefore swells readily in water (Minke and Blackwell, 1978)

2.3 Current source of chitin and its characteristics

Most of the commercialized chitins were obtained from the waste products of the crab and shrimp processing industry. Crustacean shell wastes have variation in term of properties portion depending on species and seasons (Green and Mattick, 1979). Therefore, different preparation methods were needed in preparing the chitin from the crustacean shells that depending on the species and seasons. However, commercially chitins were produced in bulk without much attention focusing on the different specificity in the chitin production that depending on the species and seasons. This is proven by data reported by US Department of Commerce in 1973 that there were over 150 000 Mt of chitin produced from crustaceans and fungi. This is the weakness of the commercially chitin as reported by Cho *et al.* (1998) that both physicochemical characteristics and functional properties of commercially available

chitin differ with products. Thus, to effectively utilize chitin as a functional ingredient, relationships between the functional properties and characteristics of chitin products must be constantly monitored for proper quality control.

2.4 Alternative sources for chitin

Chitin is known to be the major component of the insect cuticle. Chitin content in the insect constitutes up to 40% of the exuvial dry mass depending on the insect species and different cuticle types (Kramer and Koga, 1986). Chitin is found in the exo- and endocuticle or in the newly secreted, unsclerotized procuticle but not in the epicuticle, the outermost part of the integument (Andersen, 1979). It functions as light but mechanically strong scaffold material and is always associated with cuticle proteins that mainly determine the mechanical properties of the cuticle. Insects possess enormous biodiversity and represent 95% of the animal kingdom. They can offer a tremendous potential as a natural resource for chitin and chitosan production. Therefore, insects can be used as an alternative source of chitin and, consequently, of chitosan.

2.5 Applications of chitin

Chitin has poor solubility and this has becoming the major limiting factor in its utilization and investigation of its properties and structure. However, various applications of chitin and modified chitins have been reported such as raw materials for man-made fibres. A study conducted by Dutta *et al.* (2004) reported that wound dressings made of chitin and chitosan fibres can accelerate wound healing by about 75%. Besides than help in medical application, chitin and chitosan fibres do have

potential applications in wastewater treatment by removing the heavy metal ions through chelation. Chitin also reported to be applied in cosmetic uses whereby chitin, chitosan and their derivatives can be used in hair care, skin care and oral care. Both chitin and chitosan can be also used in toothpaste, mouthwashes and chewing gum that chitin helps in freshen breath and prevent formation of plaque and tooth decay.

Other studies on health aspect were also continued for the application of chitin. The study applied on various animal species. Research investigated by Vahedi and Ghodratizadeh (2011) reported that supplementing total of 50 rainbow trout with crab shell chitin (Sigma) diet 10, 25 or 50 mg/kg enhances rainbow trout immune activity through the non-specific modulation of haemolytic complement activity and leukocyte respiratory burst activity. Other study conducted in gilthead seabream by Esteban *et al.* (2001) on the effects of injecting chitin particles intravenously and intraperitoneally on the innate immune response reported that fish that had been intraperitoneally injected showed increased humoral and cellular immune responses. Natural haemolytic complement activity increased from 5 days post-injection although no statistically significant differences were observed. Respiratory burst and phagocytic activities peaked at 3 and 5 days post-injection, respectively, while cytotoxic activity had increased by 3 days post-injection and remained high until 10 days post-injection. While in study done by Hossain and Blair (2007) on the chitin utilisation by broilers and its effect on body composition and blood metabolites revealed that triglyceride concentrations in liver and breast meat were significantly reduced by chitin inclusion and serum cholesterol and triglycerol concentrations

were also reduced significantly. However, no significant differences in carcass yield and treatment effects on weight gain or feed efficiency were found.

2.6 Growth promoter

Good health status is the only aim among the farmers in the livestock production. Good health status includes good growth performances and survivability. So far, sub-therapeutic dosages of antibiotics is the only miracle reported to increase growth rate and feed efficiency of poultry and other livestock as a result of improved gut health, nutrient utilization and improved feed conversion efficiency (Visek, 1978; Landy *et al.*, 2011). Current commercialized broiler feed have already provided with these sub-therapeutic antibiotics. Improved growth performance will benefit the producer and also consumer.

Chitin shows variety results in improving growth performance of livestock. Study conducted by Nakagawa *et al.* 2011 reported that 10% supplementation of crustacean chitin in the diet fed to 0-year old ayu (*Plecogglossus altivelis*), black sea bream (*Acanthopagrus schlegelii*), and red sea bream (*Pagrus major*) showed improvement in growth, feed efficiency, and survival rate of those wild fish. In addition, according to Abdul Razak *et al.* (2012) chicks fed with house cricket meal (HCM) diet which contained chitin recorded significant higher weight gain than those chicks fed soy bean meal (SBM) but slightly lower than those chicks fed fish meal (FM) diet and the differences were not significant. Dutta *et al.* 2004 also reported that, incorporation of chitin in poultry feed at level of 0.5% decreased the feed consumption ratio and increases body weight by 12% as compared to birds with chitin free diet. However in the project done by Hossain and Blair (2007) reported

that no significant treatment effect of diet on weight gain or feed efficiency in the three weeks old broilers supplemented with chitin with 0, 25, 50, and 75 g chitin per kg diet. All the chitin used in the previous studied were obtained from the commercial crustacean shell wastes.

2.7 Selected clinical biochemistry parameters

Blood parameters are the other measurements for health status other than growth performance. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in blood parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature (Landy *et al.,* 2011). Adeyemo *et al.*, 2013 also reported that in 1963, World Health Organisation (WHO) has recommended for using blood parameters on medical and nutritional assessments, because blood contains several metabolites that provides useful information on nutritional status and clinical investigation of an individual.

Effect of chitin on blood parameters have shown various results. Hossain and Blair (2007) reported that chitin as feed supplement in broilers has resulted in significantly reduction in serum cholesterol and triglyceride values. No other studies related to the effect of chitin on blood parameters of poultry or other animals have been conducted.

Chapter 3

MATERIALS AND METHODS

This research was conducted in accordance with guidelines for regulation of animal experimentation of Universiti Putra Malaysia (UPM), Malaysia.

3.1 Insect chitin (IC) preparation

Insect chitin (IC) was prepared according to method of Majtan *et al.* (2007). Live house cricket (HC) about 8 weeks old were sacrificed by placing them in the freezer at -20ºC for 48 hours. After that, it were washed with clean tap water and dried in the oven at 60ºC for 72 hours. HC were then freeze dried about 1 minute before being grounded into meal form by grinding machine. The HC meal were then soaked into hydrochloric acid (HCl) for 24 hours. Each 20g of HC meal were treated with 5 litres of 1M HCl. After demineralization process, the HC meals were washed with tap water until the pH became neutral and dried in the oven at 60°C for 24 hours. The HC meal were then boiled with 1M sodium hydroxide (NaOH) for another 24 hours at 80ºC to deproteinised the sample. After deproteinization process, the meal were rinsed with tap water until the pH became neutral and then the whole chitin products were dried in oven at 70°C for 24 hours before ready to be fed for the chicks. In principle, the acidic step is done to remove the minerals and the hydroxide step is done to remove the cuticle protein from the crickets. The final product from this processing procedure is called chitin.

3.2 Experimental animals

In this study, a total of 36 Ross breed broiler chickens aged 21-days were used with an average of 774.58 g. the bird are divided into three treatment groups and each group contained 12 birds. Each treatment group is divided into three replicates consisting of 4 birds per replicate. All birds were reared on the wire mesh floor cages for three weeks. On arrival, the birds were weighed and randomly assigned accordingly to each treatment. The birds were provided with continuous supply of clean fresh water and the feeding regime was done at 9 a.m. every morning. No temperature, humidity and ventilation control were monitored.

3.3 Treatment diets and experimental period

The experiment consisted of 3 treatment groups with control group received only basal diet of commercialized broiler starter (CBS), the second group receive CBS plus 1g IC per kg of diet, and the third group received with CBS plus 2g IC per kg of diet. Chicks were fed once daily and had free access to clean water throughout the entire 21-days of experimental period. Feed intakes of broilers were weighted daily which is based on limit feeding 40.5 g/d. Average daily gain (ADG) and feed conversion were calculated at the end of the experimental period.

3.4 Blood collection and analysis

Blood sample were taken on day 0, 7, 14 and 21 of experimental period for clinical chemistry; haematology and serum biochemistry analysis. Two millilitres of blood were obtained by vena puncture on the left wing vein from all chicks weekly for blood chemical analysis. The blood samples were transferred in two different blood

tubes which were plain blood tube and EDTA blood tube, and then transferred straight to the laboratory. For haematological analysis, blood in EDTA tube is analysed by using CELL-DYN 3700 analyser for complete blood count (CBC) and the remaining blood were used for haematocrit and plasma protein analysis manually. The blood samples were also prepared on glass slide and painted by Wright's stain for WBC count manually. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under light microscope with oil emulsion then heterophil to lymphocyte ratio were calculated and recorded. Blood in plain blood tubes were centrifuged at 1500 g for 10 min. The serums were removed and stored at -20°C until analysed using an auto-analyzer (BT 3000 plus, Biotechnica, Rome, Italy).

3.5 Parameters measured

Parameters recorded in this study include feed intake, live weight changes, average daily gained and feed conversion ratio.

The haematology analysis included red blood cell count, white blood cell count; lymphocytes, neutrophils and eosinophil, haematocrit, haemoglobin, and heterophil to lymphocytes ratio. While for serum biochemistry, the analysis includes albumin to globulin ratio, total protein, albumin, total cholesterol, and triglycerides.

3.6 Experimental design and data analysis

The experimental design for this study is completely randomised design. Data was then subjected to ANOVA using general linear model (SPSS 20th edition).

Chapter 4

RESULT

4.1 Chemical composition of experimental diets

The chemical composition of the experimental basal diet is shown in Table 1. The commercial basal diet contained 21% crude protein, 5% fat and crude fibre, and 8% ash. The main feed ingredients in the basal diet are corn, soybean meal, grain by products, dicalcium phosphate, and trace minerals mixture. The metabolised energy consumed by the birds from this diet is 3,200 ME, kcal/kg.

Table 1. Chemical composition of experimental basal diets

Chemical composition (Dry matter basis)	(%)
Crude protein	21.0
Crude fibre	5.0
Crude fat	5.0
Moisture	13.0
Ash	8.0
Calcium	0.8
Phosphorus	0.4

4.2 Growth performance

Table 2 and Table 3 show the mean daily feed intake, live weight changes, average daily gain (ADG), and feed conversion ratio (FCR) of the broiler chickens fed basal diet containing different levels of IC. The control group recorded higher ADG that the groups containing treatments even though the daily feed intake were similar among treatments. However, the differences were not significant. The results also showed that IC supplemented treatment groups had poor-to-gain ratio as compared to the control group. The treatment group supplemented with 2g IC recorded slightly

higher ADG and FCR than treatment group receiving 1g IC but the differences were not significant.

The final live body weight of all birds in the experiment range from 1240.5 to 1305.2g at the end of 21 days of experimental period. Birds in treatment 2 showed significantly higher live weight changes in week 1 and week 2 as compared to other treatments on the same period.

Table 2. Effect of IC supplementation on the growth performance parameters

Treatments	Performance Parameters			
	Live weight gain, (g)	*ADG, (g)	Feed Intake, (g/d)	*FCR
Control	505.58[a]	24.08[a]	40.50[a]	1.68[a]
Treatment 1 (1g IC/kg diet)	468.36[a]	22.35[a]	40.50[a]	1.81[a]
Treatment 2 (2g IC/kg diet)	494.75[a]	23.56[a]	40.50[a]	1.72[a]

[a, b] Mean with different superscript within rows has significant difference (P<0.05)
*ADG = average daily gain, FCR = feed conversion ratio

Table 3. Effect of IC supplementation on weekly growth performances of broiler chickens

Treatments	Weekly live weight changes			
	Day 0	Day 7	Day 14	Day 21
Control	742.17[a]	937.50[a]	1086.75[a]	1247.75[a]
Treatment 1 (1g IC/kg diet)	835.82[a]	1008.91[b]	1158.45[b]	1305.18[a]
Treatment 2 (2g IC/kg diet)	745.75[a]	937.25[a]	1069.08[a]	1240.50[a]

[a, b] Mean with different superscript within rows has significant difference (P<0.05)

4.3 Blood biochemistry parameters

The effects of IC supplementation on blood characteristics in broiler chickens are shown in Table 4. The result shows that control group has the higher value for RBC, PCV, and Hb as compared to other treatment groups. Birds receiving 2g IC per kg basal diet has the lower WBC count; 61.10 $x10^9$/L when compared to birds receiving 1g IC in their diet but has the higher WBC count. The lowest H/L value is recorded in birds from control group, followed by birds in treatment 2 group and treatment 1 group. However all the differences recorded are not significant.

Table 4. Effect of IC supplementation on blood parameters of broiler chickens

Treatments	Blood parameters				
	*RBC ($x10^9$ /L)	*PCV (%)	*Hb (g/L)	*WBC ($x10^9$ /L)	*H/L
Control	2.33[a]	27.00[a]	10.78[a]	63.03[a]	1.39[a]
Treatment 1 (1g IC/kg diet)	2.30[a]	25.67[a]	10.67[a]	83.27[a]	3.08[a]
Treatment 2 (2g IC/kg diet)	2.20[a]	26.67[a]	9.74[a]	61.10[a]	1.99[a]

[a, b] Mean with different superscript within rows has significant difference ($P<0.05$)
*RBC-red blood cells, PCV-packed cell volume, Hb-haemoglobin, WBC-white blood cells, H/L-heterophils to lymphocytes ratio.

Table 5 shows the effect of IC supplementation on selected serum biochemistry parameters on broiler chickens. The value of TP recorded was highest in treatment 2g IC followed by treatment 1g IC and control group but the difference was not significant. All other serum parameters recorded among treatments were similar.

Table 5. Effect of IC supplementation on serum parameters of broiler chickens

	Serum parameters				
Treatments	*TP (g/L)	Albumin (g/L)	*A:G	Cholestrol (mmol/L)	*TG (mmol/L)
Control	39.87[a]	15.43[a]	0.64[a]	3.96[a]	0.25[a]
Treatment 1 (1g IC/kg diet)	52.17[a]	15.27[a]	0.43[a]	3.51[a]	0.23[a]
Treatment 2 (2g IC/kg diet)	46.83[a]	16.23[a]	0.64[a]	4.42[a]	0.36[a]

[a, b] Mean with different superscript within rows has significant difference ($P<0.05$)
*TP-total protein, A:G-albumin to globulin ratio, TG-triglyceride

Chapter 5

DISCUSSION

Based on the result recorded in Table 2 on the effect of IC supplementation on the growth performance, there were no significant different ($p > 0.05$) between the treatment groups. This result is similar with the finding of Hossain and Blair (2007) who reported that no significant treatment effect of diet on weight gain or feed efficiency in the three weeks old broilers supplemented with chitin with 0, 25, 50, and 75 g chitin per kg diet in their feed. However in study conducted by Hossain and Blair (2007), the chitin source was obtained from crustacean shell waste and it chemical structure is different from the chemical structure of IC which is used in the present study. Further study is needed to verify the differences in chemical structure of IC in order to understand how IC works in the digestive system. Weekly weight gain was significantly higher in chicks fed with 1g IC than the other two treatment groups. However, the significant value was only recorded on day 7 and 14 of the experimental period but not on week 3. This result may be influenced by the one mortality recorded in that treatment group which affect the sample number during statistical analysis.

Insect chitin also failed to induce alteration in selected haematology and serum biochemistry parameters. Result in Table 4 shows no significant differences ($p > 0.05$) in between treatment groups on the haemogram values. Blood contains several metabolites that provide useful information on nutritional status and clinical investigation of an individual (Adeyemo *et al.*, 2013). Based on the result, the birds

seem to be healthy without any marked alteration on the parameters when compared between treatment groups.

Result recorded in Table 5 for the effect of IC supplementation on serum parameters of broiler chickens recorded no significant differences ($p > 0.05$) in between treatment groups on the serum biochemistry values. This result is opposite to the finding of Hossain and Blair (2007) who supplementing seafood chitin in broiler feed resulted in significantly reduction ($p < 0.05$) in serum cholesterol and triglyceride values. However based on the result above, chicks fed with 1 g of IC per kg diet shows reduction in serum cholesterol and triglycerides values when compared to other two treatment groups in this study.

Many studies conducted on the applications of chitin is based from the commercialized chitin extracted from the crustacean shells. The procedures in extraction differ for each species accordingly. Different extraction procedures may also apply to chitin extraction from terrestrial organisms; which in this case from insects. According to Liu *et al.* 2012 the most common method for chitin extraction from insects involves two steps, an acidic step to remove catechols and a basic step to remove the cuticle proteins. These various chitin preparation steps may resulted in variation of the effect of IC when supplemented on broilers as stated by Cho *et al.* 1998 that both physicochemical characteristics and functional properties of commercially available chitin differ with sources.

Besides that, only a few studies have been conducted on the effect of IC especially on broiler chickens. This is proven in the statement made by Liu *et al.*, 2012 that only limited numbers of insect species have been documented to be

sources of chitin. Therefore, the current study is considered as the first study done on chitin extraction form HC. The current study lack proper guidelines on how to extract the chitin from insect and also how much chitin should be included in the broiler diet. The level of toxicity is still being assessed and therefore very minimal concentration of IC has been used in this study which probably resulted to no differences on performance and blood metabolites between animals in the treatment groups.

Chapter 6

CONCLUSION

The results from this study suggested that supplementation of IC in broiler diet did not improve their growth performances and selected clinical biochemistry parameters. The animals were not responding to IC supplementation probably due to low concentration tested in their diet. Other factors that contributed to poor response are IC not properly prepared and animals may need longer time to response to different chitin treatments. Therefore, further studies are needed especially on correct method of IC preparation and extraction, determination of chemical configuration of IC, and IC effective level of supplementation to the broiler chickens.

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Appendices

Figure 3 Live house cricket (HC) about 8 weeks old on arrival

Figure 4 HC after sacrified in in the freezer at -20^{o}C for 48 hours

Figure 5 Grinder machine used to grind the HC into meal form

Figure 6 Demineralization process of HC treated with 5 litres of 1M HCl for 24 hours.

Figure 7 Deproteinization process of HC meal with NaOH at 80^{o}C for 24 hours**.**

Figure 8 IC ready to be fed to the birds.