UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CHITIN AND CHITOSAN EXTRACTED FROM HOUSE CRICKET (Brachytrupes portentosus Lichtenstein) ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS

IBITOYE E. BUSAYO

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By

IBITOYE E. BUSAYO

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

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DEDICATION

This Thesis is dedicated to the Almighty God, the giver of life and all good things, who has given me good health and life to pursue this degree. To my lovely family: my beautiful and ever supporting wife (Mrs. Adeola Modupe Ibitoye) and my children (Dorcas, Berenice and Neriah).

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF CHITIN AND CHITOSAN EXTRACTED FROM HOUSE CRICKET (*Brachytrupes portentosus* Lichtenstein) ON PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS

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Chairman : Lokman Hakim Idris, PhD
Faculty : Veterinary Medicine

By 2022, the worth of feed additive in the global market would be over US$11 billion. Chitin (CT) and chitosan (CS) are potential feed additives and are biologically beneficial. However, studies have concentrated mainly on crustacean CT and CS; data on their effects on growth performance and immune response in poultry are inconsistent, while information on the use of CT and CS from other sources in poultry production is unavailable. The present study therefore aims at evaluating the effects of dietary cricket and shrimp CT and CS on growth performance and immune response in broiler chickens. Chitin and chitosan were extracted from about 8-week old house cricket (*Brachytrupes portentosus*), using a chemical method, whereas shrimp CT and CS were commercially obtained. Then, both the cricket chitin (CCT) and chitosan (CCS) and shrimp chitin (SCT) and chitosan (SCS) were physicochemically analyzed for comparison. To study the effect of dietary CCT, CCS, SCT and SCS on the growth performance and immune response of broiler chickens, a total of 150 day-old male Cobb500 broiler chicks were randomly allotted into one of the five dietary treatments with three replicates. Birds in various treatments were fed as follow: treatments 1 (T1) basal diet alone; treatment 2 (T2) 0.5 g/kg diet CCT with basal diet; treatment 3 (T3) 0.5 g/kg diet CCS with basal diet; treatment 4 (T4) 0.5 g/kg diet SCT with basal diet; while treatment 5 (T5) were fed 0.5 g/kg diet SCS with basal diet. To evaluate the effect of the treatments on the gut health, the structural morphology of the villus height (VH) and crypt depth (CD) of the jejunum and duodenum were evaluated. In addition, relative mRNA expression of oligopeptide transporter (PepT1), excitatory amino acid transporters 3 (EAAT3), Na^+-dependent glucose and galactose transporter (SGLT1) and Na^+-independent fructose transporter (SGLT5) were determined from the jejunum by real-time PCR. Furthermore, in order to evaluate the immune response of the experimental birds to dietary CT and CS, weights of Bursa of Fabricius (BF) and spleen were evaluated and the relative expression of Toll-like receptor-4 (TLR-4), Toll-like receptor-15 (TLR-15), interleukin 1-β (IL-1β) and inducible nitric oxide synthase (iNOS) from the spleen of chickens were studied using real-time PCR. This study revealed that house cricket is a potential source of good quality CT and CS and that CCT and CCS are similar to SCT and SCS respectively. However, CCT is of purer and of better quality than the SCT. The feeding trials showed that dietary CT and CS at 0.5 g/kg have a significant ($p<0.05$) effect on body weight (BW), body weight gain (BWG) and body weight adjusted for gizzard and abdominal fat. The BW and BWG of experimental birds in T2 (CCT) differ non-significantly ($p>0.05$) from the control, while the BW and BWG of the broilers in T3 (CCS) and T5 (SCS) decreased significantly ($p<0.05$). Also, abdominal fat accumulation in the chickens under T2 (CCT) and T5 (SCS) reduced significantly ($p<0.05$). Study of the haemato-biochemistry of broiler chickens showed that broilers under T5 (SCS) were markedly stressed, while those in T4 (SCT) in addition to being stressed were also anaemic with marked thrombocytopenia. More so, eosinophilia was observed in birds under T3 (CCS) and T5 (SCS). However, T2 (CCT) had no statistical adverse effect on haemopoiesis, while it also lower serum cholesterol and triglycerides of experimental broiler chickens. At day 21, dietary CT and CS had no statistical ($p>0.05$) effect on the index immune organs, while T4 (SCT) significantly reduced the weights of BF and spleen of broiler chickens at day 42. Nevertheless, broiler of T2 (CCT) and T5 (SCS) had improved BF and spleen similar to the control. The mRNA expressions of TLR-4, TLR-15, IL-1β and iNOS were significantly ($p<0.05$) impacted in this study. At day 21 and relative to the control (T1), T5 (SCS) significantly up-regulated the gene expression of TLR-4 and IL-1β, while other treatments (T2, T3 and T4) non-significantly ($p>0.05$) down-regulated them. In addition, T2 (CCT), T4 (SCT) and T5 (SCS) numerically increased the expression of TLR-15, whereas, T3 (CCS) decreased it. More so, these treatments (T2, T3, T4 and T5) significantly decreased the gene expression of TLR-4, TLR-15 and iNOS at day 42. Treatments 3 (CCS), T4 (SCT) and T5 (SCS) significantly ($p<0.05$) improved the jejunal CD and VH:CD at day 21, while they were significantly improved by T4 (SCT) at day 42. The gut morphology of broiler chickens under T4 (SCT) was significantly ($p<0.05$) better than those of T2 (CCT). Moreover, at day 21 and relative to the control, dietary CT and CS significantly ($p<0.05$) down-regulated the relative gene expression of PepT1, EAAT3, SGLT1 and SGLT5, while only dietary CS (T3 and T5) at day 42 significantly ($p<0.05$) decreased the gene expression of PepT1, EAAT3, SGLT1 and SGLT5. Therefore, chickens in T2 (CCT) had a similar growth performance and immune response with the control, but better than those of T3 (CCS), T4 (SCT) and T5 (SCS). In addition, birds of T2 had the lowest abdominal fat accumulation in the chickens.

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

KESAN KITIN DAN KITOSAN YANG DIEKSTRAK DARIPADA CENGKERIK RUMAH (*Brachytrupes portentosus* Lichtenstein) TERHADAP PENINGKATAN DAN TINDAKBALAS KEIMUNAN AYAM PEDAGING

Oleh

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Menjelang tahun 2022, nilai makanan tambahan dalam pasaran global dijangkakan melebihi US$11 bilion. Kitin (CT) dan kitosan (CS) merupakan makanan tambahan yang berpotensi dan mempunyai faedah secara biologikal. Namun demikian, kajian masa kini hanya tertumpu kepada CT dan CS dari krustacea, data yang tidak konsisten mengenai kesan CT dan CS terhadap prestasi tumbesaran, tindakbalas keimunan pada ayam dan maklumat mengenai penggunaan CT serta CS selain dari krustacea dalam bidang penternakan ayam yang sukar diperolehi. Kajian ini dijalankan bertujuan untuk menilai kesan dari amalan pemakanan CT dan CS yang diekstrak daripada cengkerik rumah dan komersial (udang) terhadap prestasi tumbesaran dan tindakbalas keimunan ayam pedaging. Kitin dan kitosan diekstrak secara kaedah kimia daripada cengkerik rumah (*Brachytrupes portentosus*) yang berumur 8 minggu manakala CT dan CS udang diperolehi secara komersial. Seterusnya, kitin (CCT) dan kitosan (CCS) cengkerik, kitin (SCT) dan kitosan (SCS) udang dibandingkan melalui analisa fisikokima. Untuk kajian kesan pemakanan CCT, CCS, SCT dan SCS terhadap prestasi tumbesaran dan tindakbalas keimunan, ayam pedaging jantan Cobb500 berumur 150 hari telah diberi satu daripada lima diet pemakanan secara rawak dengan tiga replikat. Ayam kajian telah diberikan pemakanan seperti berikut: rawatan 1 (T1) diberi diet asas (kawalan); rawatan 2 (T2) campuran 0.5g/kg CCT dan diet asas, rawatan 3 (T3) campuran 0.5 g/kg CCS dan diet asas, rawatan 4 (T4) campuran 0.5g/kg SCT dan diet asas manakala rawatan 5 (T5) campuran SCS dan diet asas. Untuk kajian kesan rawatan diet terhadap kesihatan usus pula, struktur morfologi kepanjangan vilus (VH) serta kedalaman krip (CD) jejunum dan duodenum dianalisa. Sebagai tambahan, ekspresi mRNA relatif bagi pembawa oligopeptida (PepT1), pembawa asid amino baik 3 (EAAT3), pembawa galaktosa dan glukosa bebas Na^+ (SGLT1) dan pembawa fruktosa bebas Na^+ (SGLT5) dikenalpasti dari jejunum menggunakan kaedah PCR masa-nyata. Selain itu, untuk mengenalpasti keupayaan tindakbalas keimunan ayam kajian terhadap diet CT dan CS, berat bursa Fabricius

(BF) dan limpa dianalisa, ekspresi relatif bagi reseptor-4 Toll (TLR4), reseptor-15 Toll (TLR15), interleukin 1-β (IL-1β) dan aruhan oksida nitrik sintase (iNOS) dari limpa ayam juga dianalisa melalui teknik PCR masa-nyata. Hasil kajian menunjukkan cengkerik rumah merupakan sumber CT dan CS yang berkualiti baik di mana CCT serta CCS masing-masing menyamai SCT dan SCS. Namun begitu, CCT adalah lebih tulen dan berkualiti lebih baik berbanding SCT. Pemberian makanan percubaan menunjukkan diet CT dan CS pada 0.5g/kg memberikan kesan yang signifikan ($p<0.05$) terhadap berat badan (BW), peningkatan berat badan (BWG) dan berat badan yang disesuaikan untuk gizi dan lemak bahagian perut. Bacaan BW dan BWG dalam kumpulan rawatan T2 (CCT) tiada perubahan secara signifikan ($p<0.05$) berbanding kawalan manakala BW dan BWG dalam kumpulan T3 (CCS) dan T5 (SCS) berkurang secara signifikan ($p<0.05$). Pembentukan lemak bahagian perut pada kumpulan ayam T2 (CCT) dan T5 (SCS) berkurang secara signifikan ($p<0.05$). Kajian hemato-biokimia terhadap ayam pedaging menunjukkan kumpulan T5 (SCS) adalah dalam keadaan tekanan manakala kumpulan T4 (SCT) mula mengalami tekanan anemik dengan tanda trombosiptopenia. Didapati juga, eosinofilia berlaku dalam ayam pedaging kumpulan T3 (CCS) dan T5 (SCS). Namun, kumpulan T2 (CCT) tidak menunjukkan kesan sampingan hemopoisis, mempunyai kadar trigliserida dan kolestrol serum yang rendah. Pada hari ke-21, diet CT dan CS tidak menunjukkan kesan statistik ($p<0.05$) pada indeks organ keimunan manakala kumpulan T4 (SCT) telah mengurangkan berat BF serta limpa ayam pada hari ke-42. Walau bagaimanapun, ayam dalam kumpulan T2 (CCT) dan T5 (SCS) meningkatkan bacaan BF serta limpa, menyamai kumpulan kawalan. Ekspresi mRNA bagi TLR-4, TLR-15, IL-1β dan iNOS memberikan impak yang baik dalam kajian ini, secara signifikannya ($p<0.05$). Pada hari ke-21 dan relatif terhadap kawalan (T1), T5 (SCS) secara signifikannya telah meningkatkan pengekpresan gen TLR-4 dan IL-1β manakala dalam rawatan T2, T3 dan T4 adalah tidak singnifikan ($p>0.05$). Kumpulan T2 (CCT), T4 (SCT) dan T5 (SCS) meningkatkan pengekspresan TLR-15 manakala berkurang dalam T3 (CCS). Rawatan 3 (CCS) mengurangkan pembebasan TLR-4 secara signifikan ($p<0.05$) manakala pembebasan IL-1β telah direndahkan secara signifikan ($p<0.05$) dalam kumpulan T3 dan T4 pada hari ke-21. Rawatan T2, T3, T4 dan T5 telah merendahkan pengekspresan gen TLR-4, TLR-15 dan iNOS secara signifikan pada hari ke-42. Rawatan 3 (CCS), T4 (SCT) dan T5 (SCS) secara signifikan ($p<0.05$) telah meningkatkan CD jejunum dan VH:CD pada hari ke-21 serta telah dipertringkatkan secara signifikan oleh kumpulan T4 (SCT) pada hari ke-42. Morfologi usus ayam dari kumpulan T4 (SCT) adalah lebih baik secara signifikan ($p<0.05$) berbanding dalam kumpulan T2 (CCT). Selain itu, pada hari ke-21, diet CT dan CS secara signifikannya ($p<0.05$) telah menurunkan pengekspresan gen relatif PepT1, EAAT3, SGLT1 and SGLT5 berkadaran dengan kumpulan kawalan manakala hanya diet CS (T3 dan T5) pada hari ke-42 merendahkan kadar pengekspresan PepT1, EAAT3 dan SGLT1 secara signifikan ($p<0.05$). Dapat disimpulkan bahawa tiada perbezaan yang signifikan ($p<0.05$) antara ayam yang diberi CT dan CS pada dos 0.5 g/kg melalui analisa pengekspresan mRNA pembawa nutrien dalam ayam. Justeru, ayam dari kumpulan T2 (CCT) menunjukkan peningkatan tumbesaran dan tindakbalas keimunan yang menyamai kumpulan kawalan tetapi lebih baik berbanding kumpulan T3 (CCS), T4 (SCT) dan T5 (SCS). Ayam dari kumpulan T2 juga menunjukkan pembentukan lemak di bahagian perut yang rendah.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

BW	Body Weight
BWG	Body Weight Gain
BF	Bursa of Fabricius
CP	Carrier protein
CGH	Chicken growth hormone
CT	Chitin
CS	Chitosan
CPC	Commercially powdered chitosan
CCT	Cricket chitin
CCS	Cricket chitosan
CD	Crypt depth
DA	Degree of Acetylation
DD	Degree of Deacetylation
FI	Feed intake
HSC	Hematopoietic stem cells
INT	Intestinal Nutrient Transporter
KFDA	Korea Food Additive Code
MS	Malaysian Standard
MW	Molecular weight
NPC	Nanopowder chitosan
PMN	Polymorphonucleate
C-2	Second carbon
ST	Serum triglycerides
SCT	Shrimp chitin

SCS	Shrimp chitosan
TSC	Total serum cholesterol
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4
T5	Treatment 5
VH	Villus height
VH:CD ratio	Villus height: Crypt depth ratio
Chol	Cholesterol
Tgl	Triglyceride

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the Study

In modern animal and poultry production, various feed additives are routinely used for the maintenance of good health and to achieve good performance indices of intensively produced poultry and livestock (European Union, 2018). The most important and widely used are the prebiotics, probiotics, phyto extracts, organic-acids and feed enzymes (Liao and Nyachoti, 2017; Khan and Elderderya, 2018). Other relatively upcoming however less commonly used feed additives are chitin (CT) and chitosan (CS). *Chiton*, a coat-of-mail shell, is the Greek root word for CT (Shahidi and Abuzaytoun, 2005). Chitin is a polysaccharide with nitrogen modification comprising of N-acetylglucosamine, linked together in beta 1, 4 glucosidal linkages. It is an analogue of cellulose; nevertheless, it has an extra amine group and a hydroxyl substitute on each monomer. It is the second most abundant bio-polysaccharide next to cellulose (LeMieux *et al.*, 2003) and widely distributed in the exoskeleton of marine invertebrates, insects and the cell wall of fungi and yeast (Tan *et al.*, 1996; Elieh-Ali-Komi and Hamblin, 2016). However, CT is not found in vascular plants, mammals and other vertebrates (Schoukens, 2009). Chitin is insoluble in many solvents because of its dense structure (Kurita, 2001), and this has led to the production of CS; a more soluble and utilizable deacetylated derivative of CT. Chitosan is an abundant polymeric product of nature and a copolymer of D-glucosamine and N-acetyl- d-glucosamine with β-(1, 4) linkage.

Chitin and CS are biologically valuable as they are biocompatible, biodegradable, non-toxic, non-antigenic, adsorption, film forming and metal chelating abilities (Rout, 2001; White *et al.*, 2002). In addition, due to their versatility, they have been useful for various fields of endeavor (Shahidi *et al.*, 1999; Ong *et al.*, 2008). It has been reported that CT and CS are a potent dietary supplement as they promote growth performance and immune response and could decrease cholesterol (Razdan and Pettersson, 1994). Some researchers have tried to isolate CT and CS from other sources, like insects, but there is a paucity of information on the *in vivo* study on their effect on poultry production and health. Therefore, this study was designed to evaluate and compare the effect of the house cricket (*Brachytrupes portentosus*) CT and CS on the production performance and immune response of broiler chickens with shrimp CT and CS.

1.2 Statement of the Problem

Despite the ubiquity and wide applications of CT and CS, currently, their production still relies solely on crustaceans (Chatterjee *et al.*, 2005; Silva *et al.*, 2007). Chitin and CS derived from crustacean varies physicochemically, possibly due to the heterogeneity in origins, the harsh chemical extraction and conversion procedures and variability in the levels of protein contamination, alongside a varying degree of acetylation (Nwe *et al.*, 2002; Tajdini *et al.*, 2010). Furthermore, the commercial CT and CS appear to have limited potential for medical and agricultural acceptability because they are limited and seasonally-dependent in supply (Kurita, 2001). In addition, they contain a high percentage of inorganic material ($CaCO_3$). More so, there is the issue of confined production locations and high processing costs associated with the chemical conversion of CT to CS (Elem and Uraku, 2017). Being valuable bioactive agents (Rout, 2001), CT and CS are under high demand in medicine (Shahidi and Abuzaytoun, 2005), surgery and tissue engineering and regenerative medicine (Shahidi *et al.*, 1999; Ong *et al.*, 2008) and this has limited its application in animal production. In 1996, the major argument on the production of CS from shrimp was the presence of shrimp antigen in the final product of CS (Tan *et al.*, 1996). Therefore, isolating CT and CS from other suitable sources could help in handling the problems on consistency in quality, production and demand.

Furthermore, publications on the impacts of CT and CS on animal performance and immune response are contradictory (Razdan and Pettersson, 1994; Chatterjee *et al.*, 2005). In a work done by Razdan and Pettersson (1994) and Zhou *et al.* (1999), dietary CS significantly depressed body weights and diet intakes, which lead to poorer feed efficiency, decreased concentrations of plasma lipid, nutrient digestion in the duodenum, lowered ileal digestibility of crude fat, organic matter and non-starch polysaccharide residues and reduced caecal short-chain fatty acid (SCFA) concentrations when compared with control ration. Contrarily, in a study by Silva *et al.* (2007), broiler chickens fed a low dietary CS supplementation (0.5 – 1 g/kg), had a growth performance better than the control birds. Tajdini *et al.* (2010), however, observed that higher (30.0 g/kg) dietary CS decreased the concentrations of plasma lipid but depressed growth of broiler chickens. In contrast, Nwe *et al.* (2002) concluded that at an inclusion level of 5.0 g/kg and above, CS had no beneficial impact on the growth of bird. In another report, it was concluded that CS could numerically increase growth in weaned piglets and the system behind this may be due to the rise in the concentration of growth hormone and enhancement in the structural morphology of the small intestines (Xu *et al.*, 2013). From the foregoing, it is not categorical which of the CT or CS really improve production performance of livestock and poultry. Furthermore, their mode of action is yet to be completely unraveled and the effect of CT and CS from house cricket on broiler growth response has also not received any good attention.

In the production of livestock and poultry, diseases are a major concern as they mitigate the production of adequate animal-derived foods (meat, milk, eggs) (FAO, 2008) and the availability of manure for crop production. This has led to an upward trend in the global use of antibiotics and various antimicrobials in poultry and livestock production industry for the treatment and prevention of infectious disease (Roberts, 2008) at sub-therapeutic levels in feeds. However, this practice has led to the drug residual effect and antibiotic resistance. Therefore, many countries have banned the use of antibiotics in livestock and are looking for the replacements for antibiotics. Hence, research efforts have been geared towards uncovering new unconventional antibiotic feed additive that can stimulate immune reactions and improve the performance of animal (Ashford *et al.*, 1977; Sandoval-Sanchez *et al.*, 2012).

Antimicrobial and immunomodulation properties of CT and CS have been demonstrated (Pusateri *et al.*, 2003; Kim *et al.*, 2014). However, some researchers argued that CS and not CT have immunostimulatory (Nishimura *et al.*, 1985; Zaharoff *et al.*, 2007), antimicrobial (Zheng and Zhu, 2003), anti-oxidative (Kim and Thomas, 2007; Yen *et al.*, 2008) and hypocholesterolemic (Ormrod *et al.*, 1998) properties. The antimicrobial activities of CS and their derivative against bacteria, yeast and fungi have been reported (Rodríguez-Vázquez *et al.*, 2015), while its activities on some foodborne pathogens has been studied (Razdan and Pettersson, 1994; Tan *et al.*, 1996; Ma *et al.*, 2001; Khajarern and Khajarern, 2002; Kobayashi *et al.*, 2002). Contrary to the findings of Zaharoff *et al.* (2007), CT derivatives have been reported to affect innate and adaptive immunities (Zhou *et al.*, 1999; Shi *et al.*, 2005). In broilers, low dietary supplementation of CS reportedly improved the weights of secondary lymphoid organs (Razdan and Pettersson, 1994; Shi *et al.*, 2005), as index of improved immune response.

Additionally, many studies have been focused on CT isolation and characterization from crustaceans and mushrooms (Shiau and Yu, 1998; Khambualai *et al.*, 2008) for commercial purposes. Recently, studies have investigated CT extracted from some insects, sponges, anthozoans and small crustaceans (*Asellus aquaticus*, *Oniscus asellus* and *Gammarus argaeus*) (El Hadrami *et al.*, 2010). These investigations focused more on CT physicochemical characterization, content and functional properties, while CS, the most valuable derivative of CT has not received enough similar study. Also, a handful of insect species has been studied in limited detail as a substitute source of CT and CS (Zheng and Zhu, 2003; Yoon *et al.*, 2007; Vargas and Gonzalez-Martinez, 2010; Wang *et al.*, 2013). Until now, there is no report of the effect of either CT or CS isolated from insect on the production performance and immune response of poultry and livestock. In addition, a study on CT and CS isolation and characterization from house cricket (*B. portentosus*) and comparative impact on the growth performance and immune response of broiler chicken are wanting.

1.3 Justification of the Study

For this study, the insect (house cricket) approach has the benefit that insects have great biodiversity and occupies about 95% of the animal kingdom (Yen *et al.*, 2008). Moreover, demineralization is easier in insect as they possess a low amount of inorganic salt (Kim and Thomas, 2007). In comparison with conventional livestock, insects can be more sustainably produced, since they have higher feed conversion efficiencies, lower water and land requirements and lower greenhouse gas emissions (Qin *et al.*, 2002). In Malaysia, formal research into CT and CS has been ongoing for no less than 20 years, with a paucity of published information of such in cricket. Insects and especially house cricket breeding farms occur in good numbers in most parts of Malaysia. Insects are rich in CT, therefore, they can offer an enormous potential as a natural resource for CT and CS production, but there have been few reports on the preparation and characterization of CT and CS from cricket.

More so, some studies have been carried out on the effective utilization of crustacean CT and CS as a feed supplement and prebiotic in animal nutrition, but they have given variable results (Razdan and Pettersson, 1994; Kobayashi *et al.*, 2002; Kobayashi *et al.*, 2006). Nonetheless, the documents on the impact of cricket CT and CS on the growth performance, carcass quality and hematology of broiler chickens are lacking. In addition, the effects of cricket CT and CS on gut morphology, immune function and gene expression of some intestinal nutrient transporters (INT) as it affects production in broiler chickens have received little attention.

The mechanism of action behind the ability of CT and CS to improve growth performance of broilers is unknown fully. However, studies have hypothesized that CT and CS could protect the mucous membrane of the stomach and improves the action of pepsin (Lv *et al.*, 2002), thus enhancing protein digestion and absorption.

1.4 Hypothesis

The physicochemical properties of cricket chitin and chitosan and their effect on growth performance and immunity of broiler chicken is the same with shrimp chitin and chitosan.

1.5 Aim and Objectives

The aim of this study is to evaluate and compare the effect of cricket CT and CS on productive performance and immune response of broiler chickens with shrimp CT and CS.

The specific objectives of the study are to:

1. Isolate CT and CS from crickets (*B. portentosus*) and physicochemically characterize and compare them with shrimp chitin (SCT) and chitosan (SCS).
2. Compare the effect of cricket and shrimp CT and CS on growth performance and carcass characteristics of broiler chickens.
3. Study the comparative impact of cricket and shrimp CT and CS on hematology and serum biochemistry of broiler chickens.
4. Assess the effect of cricket and shrimp CT and CS on immune response and lymphoid organs of broiler chickens.
5. Study the comparative effects of cricket and shrimp CT and CS on gut morphology, mRNA expression of INT and growth hormones of broiler chickens.

1.6 Limitation of the Study

Due to unavailability of large extraction vat, more time was spent on the extraction of chitin and chitosan from house cricket. In addition, due to inadequate funding other haematological parameters evaluated was scaled down.

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