ผลของสารสกัดเจลพอลิแซ็กคาไรด์จากเปลือกทุเรียน *Durio zibethinus* ต่อการตอบสนองทางภูมิคุ้มกันในไก่และปริมาณคอเลสเตอรอลในกล้ามเนื้อของไก่

นางสาวณัษฐา กิจประเทือง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

EFFECTS OF POLYSACCHARIDE GEL EXTRACTED FROM DURIAN *Durio zibethinus* FRUIT-RINDS ON IMMUNE RESPONSES IN CHICKENS AND QUANTITY CHOLESTEROL IN CHICKEN MUSCLE

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Thesis Title	EFFECTS OF POLYSACCHARIDE GEL EXTRACTED FROM DURIAN *Durio zibethinus* FRUIT-RINDS ON IMMUNE RESPONSES IN CHICKENS AND QUANTITY CHOLESTEROL IN CHICKEN MUSCLE
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ณัษฐา กิจประเทือง : ผลของสารสกัดเจลพอลิแซ็กคาไรด์จากเปลือกทุเรียน Durio zibethinus ต่อการตอบสนองทางภูมิคุ้มกันในไก่และปริมาณคอเลสเตอรอลในกล้ามเนื้อของไก่ (EFFECTS OF POLYSACCHARIDE GEL EXTRACTED FROM DURIAN *Durio zibethinus* FRUIT-RINDS ON IMMUNE RESPONSES IN CHICKENS AND QUANTITY OF CHOLESTEROL IN CHICKEN MUSCLE.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. นาตยา งามโรจนวณิชย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. สุนันท์ พงษ์สามารถ, รศ. น.สพ. ดร. นิวัตร จันทร์ศิริพรชัย, 86 หน้า

สารพอลิแซ็กคาไรด์เจล (PG) สกัดจากเปลือกของผลทุเรียน (*Durio zibethinus*) เป็นสารเพคติคพอลิแซ็กคาไรด์ที่มีฤทธิ์ต่อภูมิคุ้มกันและต้านแบคทีเรียได้ การศึกษานี้มีวัตถุประสงค์เพื่อ (1) ประเมินผลของ PG ในฐานะอาหารเสริมในด้านการเพิ่มน้ำหนัก อัตราแลกเนื้อ ผลต่อระบบภูมิคุ้มกัน การลดจำนวนแบคทีเรียโดยรวมและซัลโมเนลลาในมูลไก่ และการลดโคเลสเตอรอลในไก่เนื้อ ใช้ไก่เนื้ออายุ 1 วันจำนวน 80 ตัว แยกออกโดยสุ่มเป็น 4 กลุ่ม ไก่กลุ่มทดลองให้อาหารที่เคลือบด้วย PG ในอัตราส่วน 1, 2 และ 3 กรัมต่อ 100 กรัมอาหารไก่ ตามลำดับ และไก่กลุ่มควบคุมให้อาหารพื้นฐานที่ไม่มี PG เป็นเวลา 42 วัน พบว่า PG ไม่มีผลแตกต่างอย่างมีนัยสำคัญทางสถิติของการเพิ่มน้ำหนักไก่และอัตราแลกเนื้อในกลุ่มทดลองและกลุ่มควบคุม ไก่กลุ่มทดลองที่ให้อาหารเคลือบด้วย PG ในอัตราส่วน 3 กรัมต่อ 100 กรัมอาหารไก่มีระดับภูมิคุ้มกันต่อโรคนิวคาสเซิลและเบอร์ซาอักเสบติดต่อที่สูงกว่าไก่กลุ่มอื่นๆอย่างมีนัยสำคัญทางสถิติ (P<0.05) ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของอัตราส่วนของค่าเฮทเทอโรฟิลล์ต่อลิมโฟไซต์ระหว่างไก่กลุ่มทดลองที่ได้รับอาหารที่เคลือบด้วย PG และกลุ่มควบคุมที่ให้อาหารพื้นฐานที่ไม่มี PG พบว่าในสัปดาห์ที่ 6 ไก่กลุ่มทดลองที่ได้รับ PG มีจำนวนแบคทีเรียโดยรวมในมูลไก่สดลดลง 81-97% อย่างมีนัยสำคัญ (P<0.05) เมื่อเทียบกับไก่กลุ่มควบคุมที่ไม่ได้รับ PG ปริมาณเชื้อซัลโมเนลลาลดลงอย่างมีนัยสำคัญทางสถิติ และไม่พบโคโลนีของเชื้อซัลโมเนลลา ในสัปดาห์ที่ 6 ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณโคเลสเตอรอลในพลาสมา ขณะที่ไก่กลุ่มที่ได้รับอาหารที่เคลือบด้วย PG มีปริมาณโคเลสเตอรอลในเนื้ออกไก่ต่ำกว่าไก่กลุ่มที่ไม่ได้รับ PG อย่างมีนัยสำคัญ (P<0.05) (2) ประเมินผลของ PG เพื่อเสริมประสิทธิภาพของวัคซีนชนิดเชื้อตายที่ผลิตขึ้นเองต่อโรคนิวคาสเซิล ทำการทดลองในไก่เนื้อ 168 ตัว กลุ่มทดลองให้วัคซีนชนิดเชื้อตายที่มี PG ในอัตราส่วน 0.5, 1.0 และ 1.5 กรัมเปอร์เซ็นต์ ตามลำดับ ทำการทดลองเป็นเวลา 55 วัน พบว่าไก่กลุ่มควบคุมลบซึ่งไม่ได้รับวัคซีนและไม่มีการป้อนเชื้อพิษนิวคาสเซิล ไก่กลุ่มควบคุมบวกซึ่งไม่ได้รับวัคซีนแต่มีการป้อนเชื้อพิษนิวคาสเซิล ไก่กลุ่มทดลองที่ให้วัคซีนชนิดเชื้อตายที่มี PG ในอัตราส่วน 0.5 และ 1.0 กรัมเปอร์เซ็นต์และไก่กลุ่มที่ได้รับวัคซีนทางการค้าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติของอัตราแลกเนื้อ สัปดาห์ที่ 4 ระดับของภูมิคุ้มกันต่อโรคนิวคาสเซิลระหว่างไก่กลุ่มควบคุมลบ ไก่กลุ่มควบคุมบวก ไก่กลุ่มทดลองที่ให้วัคซีนชนิดเชื้อตายที่มี PG ในอัตราส่วน 0.5, 1.0 และ 1.5 กรัมเปอร์เซ็นต์ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับไก่กลุ่มที่ได้รับวัคซีนทางการค้า ในสัปดาห์ที่ 5 พบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของระดับภูมิคุ้มกันต่อโรคนิวคาสเซิลระหว่างไก่กลุ่มควบคุมลบ กลุ่มควบคุมบวกและไก่กลุ่มทดลองที่ให้วัคซีนชนิดเชื้อตายที่มี PG ในอัตราส่วน 0.5 และ 1.0 กรัมเปอร์เซ็นต์ แต่มีค่าต่ำกว่ากลุ่มทดลองที่ฉีดวัคซีนที่มี 1.5%PG และกลุ่มที่ได้รับวัคซีนทางการค้า จากการทดลองสามารถสรุปได้ว่าใช้ PG เป็นสารช่วยเสริมในอาหารไก่เพื่อช่วยเสริมสุขภาพในไก่ในการช่วยลดปริมาณแบคทีเรียในมูลไก่ เพิ่มการตอบสนองทางภูมิคุ้มกันของไก่และลดปริมาณโคเลสเตอรอลในเนื้อไก่ได้

ลายมือชื่อนิสิต .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

สาขาวิชา เทคโนโลยีชีวภาพ ..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

ปีการศึกษา 2555 ..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : cholesterol, dietary fiber, feed conversion ratio, polysaccharide gel

NUSTHA KITPRATHAUNG : EFFECTS OF POLYSACCHARIDE GEL EXTRACTED FROM DURIAN *Durio zibethinus* FRUIT-RINDS ON IMMUNE RESPONSES IN CHICKENS AND QUANTITY OF CHOLESTEROL IN CHICKEN MUSCLE. ADVISOR : ASSOC. PROF. NATTAYA NGAMROJANAVANICH, Ph.D., CO- ADVISOR : ASSOC. PROF. SUNUNTA PONGSAMART, Ph.D., ASSOC. PROF.NIWAT CHANSIRIPORNCHAI, D.V.M., Ph.D., 86 pp.

Polysaccharide gel (PG) from the fruit-rind of durian (*Durio zibethinus* Murr.) was studied. This study was to evaluate:(1) the effects of PG as a feed additive diet on body weight gain, fed conversion ratio (FCR), immune stimulation, total bacteria and *Salmonella* in feces and cholesterol levels in broilers. Eighty broiler chicks at one day of age were divided into 4 groups. Three experimental groups were fed a commercial diet coated with PG at 1, 2 and 3 g/100 g, respectively, and the control group was fed a commercial diet without PG. The study was performed for 42 days. Chicken weight gain and FCR in the treatment and control groups were not significantly different. The hemagglutination-inhibition (HI) antibody titers against Newcastle disease (ND) virus and ELISA antibody titers against the infectious bursal disease (IBD) virus were significantly different ($P<0.05$). The chickens fed the commercial feed added with 3 g/100 g PG revealed the highest titers against the ND and IBD antibodies than the other groups. There was no significant difference of heterophil:lymphocyte ratio between the treatment groups fed with PG and the control group fed without PG. Total bacteria counts in chicken feces were reduced and exhibited 81-97 % reduction in the experimental groups after feeding PG additive diet for 6 weeks. The *Salmonella* detected colonies were not found at week 6. Cholesterol levels in the plasma of chickens in the treatment and control groups were not significantly different. However, the cholesterol contents in the muscles of the broilers fed with 3% PG were significantly lower than the chickens fed without PG ($P<0.05$). (2) the effects of PG as a vaccine adjuvant. One hundred and sixty eight broilers were divided into 3 experimental groups. Three experimental groups were vaccinated with inactivated NDV vaccine containing PG at 0.5, 1.0 and 1.5 g/100 g vaccine; The study was performed for 55 days. The FCR in the negative control, positive control groups, the group received inactivated vaccine with 0%, 1.0% and 1.5% PG and the group were received commercial inactivated ND vaccine were not significant difference. The HI titers against ND virus showed at week 4 that HI titer in negative control, positive control and treated group injected with inactivated vaccine with 0, 0.5, 1.0 and 1.5% PG as well as commercial inactivated ND vaccine were not significant difference ($P<0.05$). At week 5, the HI titer values in negative and positive control group and treated group injected with 0.5 and 1% PG were not significant difference but significantly lower than those of group injected with 1.5% PG and commercial vaccine. The study found that the PG in diet benefited health promotion of broilers chickens in reduction fecal bacteria, immunostimulation and cholesterol reduction. PG can be used as a vaccine adjuvant to improve the immune system in chicken.

Department : Biotechnology ..... Student's Signature .....

Field of Study : Biotechnology ..... Advisor's Signature .....

Academic Year : 2012 ..... Co-advisor's Signature .....

Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

µg	= microgram
µl	= microliter
C	= degree Celsius
ELD50	= 50% chicken embryo lethal dose
ELISA	= enzyme-linked immunosorbent assay
hr	= hour
HA	= hemagglutinins
HCl	= hydrochloride
HI	= hemagglutination inhibition
L	= liter
mg	= milligram
ml	= milliliter
NaCl	= sodium chloride
NSS	= normal saline
PBS	= phosphate buffer saline
v/v	= volume/volume
w/v	= mass/volume

CHAPTER I

GENERAL INTRODUCTION

Durian is the most popular fruit in Thailand, which have been cultivated in eastern and southern Thailand. The soluble fiber (polysaccharide gel; PG) and insoluble fiber (polysaccharide fiber) in durian fruit-rind have been characterized (Girddit et al., 2001; Sithipairojsakul et al., 2001; Hokputsa et al., 2004) The polysaccharide gel composes of the long chain $\alpha(1\rightarrow4)$ linked polygalacturonan with side chains $\alpha(1\rightarrow6)$ of neutral sugars and terminal non-reducing end fructose; and short chain amylose have also found (Hokputsa et al., 2004). The insoluble polysaccharide fiber in fruit-rind composes of long chain glucan as α-cellulose (Sithipairojsakul et al., 2001; Siralertmukul et al., 2005). The polysaccharide gel swells and slowly disperses in water. Gelling property of polysaccharide gel has benefited for utilizing as pharmaceutical aids. Soluble and insoluble durian polysaccharides are useful for tableting (Umpray et al., 1990; Sithipirojsakul et al., 2002). The polysaccharide gel is resistant to acid and enzyme α-amylase hydrolysis. The polysaccharides cannot be completely digested and absorbed in GI tract. Furthermore, study by Pholdaeng and Pongsamart (2010) has reported that adding the PG component to the diet did not enhance growth promotion in shrimp, the authors suggest that a viscosity of a high PG concentration may cause interference in the rate of nutrient absorption in shrimp. High viscosity of the diet can likely retard nutrient absorption in the same way as the effect of dietary fiber (Fair et al., 1980). Too high level of PG in the diets may not be appropriate because of the retardation of nutrient absorption in the digestive tract. The toxic effects have not found in acute and subchronic toxicity studies; this study confirmed consumptive safety of durian polysaccharides (Pongsamart et al., 2001, 2002). Wound dressing preparation made of durian polysaccharide gel has advantages of wound healing, toxic skin reaction has not observed (Chansiripornchai et al., 2005). The PG has a beneficial effect in improving the immune responses of black tiger shrimp (*Penaeus monodon*)

(Pholdaeng and Pongsamart, 2010). Moreover, a preliminary study by Chansiripornchai et al. (2008) reported that adding PG to broiler chicken diet, as a feed additive, has potentially stimulated the chicken immune responses and reduced cholesterol level in the pectoral muscles of chickens. Broiler chickens are an economically important source of valuable food products because of the low investment of both time and money. Broiler production provides a faster and higher profit than the other types of chicken production. Currently, broilers are not only produced at the industrial level but also the small holding level is interested in broiler farming. Small holders serve as an important source of the food supply in remote and poor areas. Thus, the export of broiler products creates revenue for both broiler farming in the small holding and at the industrial level. An outbreak of infectious disease (such as Newcastle disease, infectious bursal disease, etc.) certainly causes severe economic damage to all levels of broiler productions. According to these concepts, the present study was designed to investigate the effect of PG as a feed additive on immunostimulation, reduction of meat cholesterol and also reduction of total bacteria in feces of chickens.

Objectives

1. To study an optimal formula of polysaccharide gel containing in feed additives that stimulates the immune response against viral infections, reduce cholesterol and reduce total fecal bacteria in chickens.
2. To study an effect of polysaccharide gel from durian fruit-rinds as co-adjuvant vaccination and its optimal formula of polysaccharide gel as co-adjuvant vaccination that stimulates the immune response against viral infections in chickens.

Expected values

1. Broiler chickens feed additive formular containing polysaccharide gel from durian fruit-rinds that having immunostimulating activity.
2. Product of low cholesterol chicken meat.
3. Reduction of antibiotic usage in chicken feed.
4. Application of PG from durian fruit-rinds as adjuvant vaccination.

CHAPTER II

LITERATURE REVIEW

1. Durian

Durian (*Durio zibethinus* Murr.) is a member of Bombacaceae family. Durian is one member that differs radically in having large seeds surrounded by fleshy arils. They are large trees, able to grow up to 40 meters in height. The leaves are evergreen, elliptic to oblong and 10-18 cm long. Apart from variants of the word "durian" in native dialects, there are few other vernacular names, though the notorious odor has given rise to the terms, "*civet cat tree"* and "*civet fruit*" in India *"stink"* in Dutch and "Thurian" in Thailand. Nevertheless, the durian is the most important native fruit of Southeastern Asia and neighboring islands (Center of agricultural information, 2004).

There are estimated to be 28 species in the genus *Durio* in Thailand. Only 5 species in addition to the durian bear edible fruits. These are *D. dulcis* Becc., *D. grandiflorus* Kost., *D. graveolens* Becc., *D. kutejensis* Becc. and ranked second to the durian in edibility; and *D. oxleyanus* Griff (Brown, 1997; Subhadrabandhu and Ketsa, 2001).

1.1 Edible Species

In addition to *D. zibethinus*, seven other species in the *Durio* genera are reported to be edible. Descriptions of these species, including *D. zibethinus,* are as follows (Brown, 1997; O'Gara et al., 2004).

Table 2-1: Descriptions of *D. zibethinus* species in Thailand.

Species	*Vernacular name*	*Distribution*
D. griffithii	*Thurian nok*	*Yala*
D. lowianus	*Chaarian, Thurian nok*	*Ranong, Trang*
D. malaccensis	*Due-yae- buu-kong, Thurian don*	*Yala, Narathiwat*
D. monsoni	*Thurian thuean, Thurian paa*	*Chumphon, Phangnga*
D. pinangianus	*Thurian*	*Yala, Narathiwat*
D. zibethinus	*Thurian*	*General*

D. zibethinus* Murr:* This is common and the most important durian species that bears fruit of very high quality and economically importance. Because it is heterogenous, it exhibits a wide range of characteristic in tree form, fruit form and aril quality amongst a population of seedling trees (Brown, 1997).

D. lowianus: This species is found in the swamp forests of southern Thailand, Peninsular Malaysia and on the island of Sumatra. It is known in Thailand as "Chaarian" (in *Ranong* province) and "*Thurian nok*" (in Trang province). The trees are occasionally cultivated because of their edible fruit. However, the round fruit only have a thin layer of fresh which is of inferior eating quality (Brown, 1997).

D. zibethinus* Murr:* "Mon thong" and "Chanee" is the durian cultivars that can be found in all center of durian cultivation in Thailand that is in central Thailand (Nonthaburi province and its environs), eastern Thailand (Rayong, Chantaburi, Trat, Prachin Buri province and its environs) and northern Thailand (Uttaradit province and its environs). The agro-climate in central and eastern Thailand is dry, so "Mon thong" and "Chanee" and particularly dominant in those regions. "Kan Yao" and "Kradum Thong" is widely grown in Uttaradit, a province situated between Bangkok and Chiangmai. The agro-climate of Uttaradit is relatively more humid than that of central and eastern Thailand (Brown, 1997).

1.2 Medicinal Uses

The flesh is said to serve as a vermifuge. In Malaysia, a decoction of the leaves and roots is prescribed as an antipyretic. The leaf juice is applied on the head of a fever patient (Morton, 1987). The leaves are employed in medicinal baths for people with jaundice. Decoctions of the leaves and fruits are applied to swellings and skin diseases. The ash of the burned rind is taken after childbirth, used to improve sexual function (Faylon, 2005). The seeds are believed to possess a toxic property that causes shortness of breath (Burkill and Haniff, 1930). The fruit is believed to have medicinal properties, restoring healthy to humans and domestic animals. Leaf, fruit and root extracts are used to reduce fever and treatment of jaundice, swelling and skin diseases (Morton, 1987).

1.3 Polysaccharide gel from *Durio zibethinus* Murr.

In the interest of environment, attempts have recently been made to use this agricultural waste as a source of valuable materials of commercial importance. A polysaccharide gel (PG) has been isolated from dried fruit-rind of durian (*Durio zibethinus* Murr. “Monthong”). A process of PG isolation was performed based on the method previously described by Pongsamart and Panmuang (1998). Previous studies dedicated by Pongsamart and co-workers have isolated the water insoluble and water soluble polysaccharides from durian rinds, which have been found potential as pharmaceutical excipients. Their applications such as a tablet binder, tablet disintegrator and gelling agent have been well reported (Umprayn et al., 1990a, 1990b; Pongsamart and Panmaung, 1998). Further investigations have also shown that the water soluble polysaccharides have antibacterial activities against certain strains gram positive and gram negative bacteria (Lipipun et al., 2002). Dressing film can be prepared from the water soluble polysaccharides and *in vivo* study demonstrated that the dressing films enhanced wound healing in pig and dog skin (Nakchat, 2003; Siripoksupkul, 2004). Polysaccharide gel have some influence on the complement system (Hokputsa et al., 2004) and also have been studies as immunomodulating activity in black tiger shrimps (Pholdaeng and Pongsamart, 2010). Moreover, a preliminary study by Chansiripornchai et al. (2008) reported that adding PG to broiler

chicken diet, as a feed additive has potentially, stimulated the immune responses and reduce cholesterol in the pectoral muscles of chickens.

1.4 Sugar composition and properties of polysaccharide gel

Pharmaceutically useful polysaccharide gel (PG) extracted from the rinds of *Durio zibethinus* are composed of pectic polysaccharide as the principal component and trace of starch as a contaminant. PG is a water soluble pectic polysaccharide, composes of sugars such as glucose (20.9%), rhamnose (4.8%), galactose (4.9%), xylose (0.4%), rhamnose (4.8%), arabinose (1.2%) and galacturonic acid (67.9%) (Hokputsa et al., 2004). The PG demonstrates a high viscosity polysaccharide.

2. Dietary fiber

Dietary fiber is a material found in or associated with food from plant, but it cannot be digested and does not provide energy (calories) or building blocks for the structural growth and maintenance of the consuming organism (Prosky and De Vries, 1992).

Most humans know that portions of the food they consume are nondigestible, that is, the feces they excrete are made up not only of waste byproducts resulting from the normal conversion of digestible foodstuffs to energy, but also of materials that passed through the alimentary system untouched by digestive enzymes. What is not realized is that, in addition to the materials that are obviously fibrous in appearance, there are food components such as pectin and β glucans, are not digested by the enzymes of the alimentary system (Greger, 1999; Eastwood and Kritchevsky, 2005).

Crude fiber is the residue left over after extensive and strenuous chemical digestion of the foods. Since this material is generally considered totally nondigestible and therefore of little benefit to humans, food nutrition tables sometimes do not report the crude fiber content of the foods. When it is reported, the main purpose of the assay is to determine the portion of the food or forage totally nondigestible by

nearly any means and thereby adjust the calculated energy (calories) content of the food accordingly (Greger, 1999; Tungland and Meyer, 2002).

Cleave (1956), noted the types of diseases observed in the residents of underdeveloped countries, these diseases states appears related to the people's diets. Residents of underdeveloped countries have diets consisting of high levels of whole plant materials and/or other whole foods. In contrast, people in industrialized nations consume very low levels of whole food or whole plant materials, a relationship between these consumption patterns and a number of diseases that the named "Fiber deficiency syndromes" that is a symptoms of having a lack of fiber in the diet. The symptoms of fiber deficiencies are associated with development of numerous health problems including constipation, hemorrhoids, colon cancer disease, obesity and elevated cholesterol levels.

Trowell, in 1972, showing the inadequacy of crude fiber figures in food nutrition tables and the importance of analyzing all the nondigestible plant cell wall materials in diet, suggested the term "dietary fiber," a term coined earlier by Hipsley (1953). This preserved the common term of fiber, associated with component nondigestibility, but allowed for a definition that would include all the significant dietary components rather than the leftover residue from the harsh crude fiber measurement technique. Trowell notes that the "hallmark of all the substances included in the term 'dietary fiber' was that they were not digested at all by the alimentary enzymes in man". Scientific definitions, for the present, dietary fiber defined as "consisting of the remnants of plant cells resistant to digestion by the alimentary enzymes of the humans" is the appropriate definition (Boerjan et al., 2003; Nugent, 2005).

2.1 Dietary fiber composition.

Currently, total dietary fiber is split into two main components: (1) insoluble dietary fiber; cellulose, hemicellulose, lignin and plant waxes and (2) soluble dietary fiber; gums, β glucans, pectins and glucomanan (Tungland and Meyer, 2002).

Pectins – Pectins or pectic polysaccharide are polymeric substances that are based on a polymer of α D-galacturonic acid, linked by linear sequences of 1,4 linkages. The main polymer has side chains that consist of sugars, galactose, glucose, rhamnose and arabinose (Figure 2-1). Pectins are primarily water-soluble, solubility being somewhat dependent on the degree of esterification of the galacturonic acid, as well as the makeup of the constituent side chains. The primary sources of pectin are citrus fruits and apples (Brody, 1999).

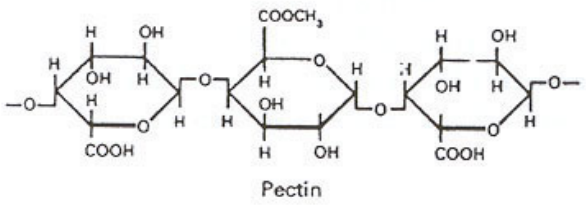


Figure 2-1: Structure of the dietary fiber pectin.

2.2 Benefits of fiber

Insoluble fiber binds water as it passes through the digestive tract, making stools softer and bulkier. Therefore, fiber, especially that found in whole grain products, is helpful in the treatment and prevention of constipation, hemorrhoids and diverticulosis. Diverticula are pouches of the intestinal wall that can become inflamed and painful. In the past, a low-fiber diet was prescribed for this condition. It is now known that a high-fiber diet gives better results once the inflammation has subsided (Anderson et al., 2009).

Low blood cholesterol levels (below 200 mg/dl) have been associated with a reduced risk of coronary heart disease. The body eliminates cholesterol through the excretion bile acid. Water-soluble fiber binds bile acids, suggesting that a high-fiber diet may result in an increased excretion of cholesterol (Abraham and Mehta, 1988). Some types of fiber, however, appear to have a greater effect than others. The fiber found in rolled oats is more effective in lowering blood cholesterol levels than the fiber

found in wheat. Pectin has a similar effect that it can lower the amount of cholesterol in the blood (Terpstra et al., 1998).

Other claims for fiber are less well founded. Dietary fiber may help reduce the risk of some cancers, especially colon cancer. This idea is based on information that insoluble fiber increases the rate at which wastes are removed from the body. This means the body may have less exposure to toxic substances produced during digestion. A diet high in animal fat and protein also may play a role in the development of colon cancer (Freudenheim et al., 1990).

High-fiber diets may be useful for people who wish to lose weight. Fiber itself has no calories, yet provides a "full" feeling because of its water absorbing ability (Hong et al., 1998).

3. Carbohydrate in diet

Carbohydrates can easily be divided into three main groups called monosaccharides, disaccharides and polysaccharides (Hong et al., 1996). The general properties of these grouping are found below.

3.1 **Monosaccharides** – These are crystalline compounds, soluble in water, sweet to taste and do not need digestion in order to be absorbed into the blood stream (Donald and Judith, 1995).

3.2 **Disaccharides** – These are crystalline compounds, water-soluble, sweet to the taste and must be digested to monosaccharides before they can be absorbed and used for energy (Dumitriu, 1996).

3.3 **Polysaccharides** – These are not water soluble and are not crystalline. They form colloidal suspensions instead of solutions. They are not sweet and must be digested before being absorbed (Smith and Montgonery, 1959). Polysaccharides are homopolysaccharides if they contain only one type of monomer and heteropolysaccharides if they contain more than one type of monomer. Homopolysaccharides are called according to their repeating unit. They may be glucans, fructans, mannans, etc (Kajiwara and Miyamoto, 1998).

The main storage forms of polysaccharides are glycogen in animal cells and starch in plant cells. Both are deposited as granules in cells. Starch can be found in one of two forms : α-amylose or amylopectin.

Amylose consists of long unbranched chains of glucose attached to each other in α(1→4) linkages as shown in Figure 2-2 (Kajiwara and Miyamoto, 1998).

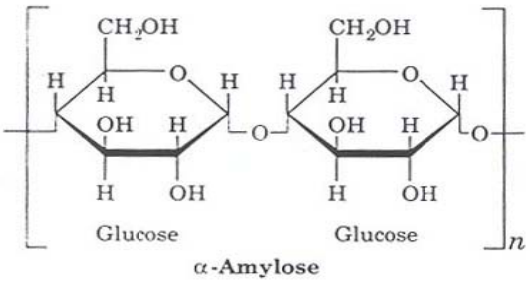


Figure 2-2: α-Amylose. Its D-glucose residues are linked by α(1→4) bonds.

Amylopectin is highly branched with 24-30 residues/branch. The chains have α(1→4) linkages but the branch points consist of α(1→6) linkages as shown in Figure 2-3 (Kajiwara and Miyamoto, 1998).

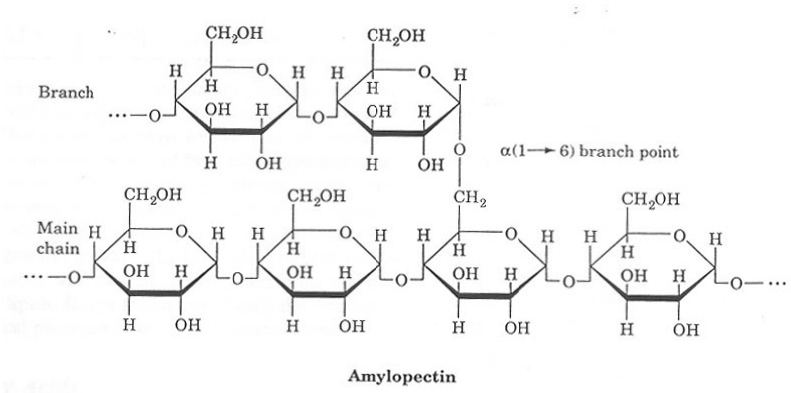


Figure 2-3: Amylopectin. Its primary structure shows one of its α(1→6) branch points.

4. Cholesterol

Cholesterol is an organic chemical substance classified as a waxy steroid of fat. It is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity.

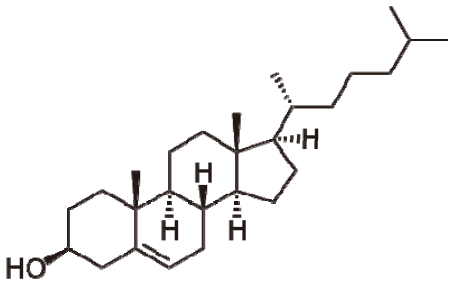


Figure 2-4: Structure of cholesterol.

In addition to its importance within cells, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D. Cholesterol is the principal sterol synthesized by animals; in vertebrates, it is formed predominantly in the liver. Small quantities are synthesized in other cellular organisms (eukaryotes) such as plants and fungi. It is almost completely absent among prokaryotes. Although cholesterol is important and necessary for human health, high levels of cholesterol in the blood have been linked to damage to arteries and cardiovascular disease (Hanukoglu, 1992).

4.1 Physiology

Since cholesterol is essential for all animal lives, each cell synthesizes it from simpler molecules. However, normal and especially high levels of fats (including cholesterol) within the blood circulation, depending on how it is transported within lipoproteins, are strongly associated with progression of atherosclerosis. However, most ingested cholesterol is esterified and non-esterified cholesterol is poorly absorbed. The body also compensates for any absorption of additional cholesterol by

reducing cholesterol synthesis. For these reasons, cholesterol intake in food has been little, if any, effect on total body cholesterol content or concentrations of cholesterol in the blood. Cholesterol is recycled. The liver excretes it in a non-esterified form (via bile) into the digestive tract. Typically, about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the bloodstream (Abraham and Mehta, 1988). Some plants make cholesterol in very small amounts. Plants manufacture phytosterols (substances chemically similar to cholesterol produced within plants), which can compete with cholesterol for reabsorption in the intestinal tract, thus potentially reducing cholesterol reabsorption. However, phytosterols are foreign to animal cells and if absorbed, accelerate the progression of atherosclerosis. When intestinal lining cells absorb phytosterols, in place of cholesterol, they usually excrete the phytosterol molecules back into the GI tract, an important protective mechanism (Abraham and Mehta, 1988).

4.2 Function

Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids. Through the interaction with the phospholipid fatty-acid chains, cholesterol increases membrane packing, which reduces membrane fluidity (Sadava et al., 2011). In this structural role, cholesterol reduces the permeability of the plasma membrane to neutral solutes (Yeagle, 1991), protons and sodium ions (Haines, 2001).

Within the cell membrane, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. Cholesterol is essential for the structure and function of invaginated caveolae and clathrin-coated pits, including caveola-dependent and clathrin-dependent endocytosis. Recently, cholesterol has also been implicated in cell signaling processes, assisting in the formation of lipid rafts in

the plasma membrane. Lipid raft formation brings receptor proteins in close proximity with high concentrations of second messenger molecules (Incardona and Eaton, 2000). In many neurons, a myelin sheath, rich in cholesterol, since it is derived from compacted layers of Schwann cell membrane, provides insulation for more efficient conduction of impulses (Pawlina and Ross, 2006).

Within cells, cholesterol is the precursor molecule in several biochemical pathways. In the liver, cholesterol is converted to bile, which is then stored in the gall bladder. Bile contains bile salts, which solubilized fats in the digestive tract and aided in the intestinal absorption of fat molecules as well as the fat-soluble vitamins, A, D, E and K. Cholesterol is an important precursor molecule for the synthesis of vitamin D and the steroid hormones, including the adrenal gland hormone's cortisol and aldosterone, as well as the sex hormone's progesterone, estrogens and testosterone and their derivatives (Incardona and Eaton, 2000). Smith (1991) indicates cholesterol may act as an antioxidant.

4.3 Dietary sources

Animal fats are complex mixtures of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence, all foods containing animal fat contain cholesterol to varying extents (Christie, 2003). Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, fish and shrimp. Human breast milk also contains significant quantities of cholesterol (Jensen et al., 1978). From a dietary perspective, cholesterol is not found in significant amounts in plant sources (Behrman and Gopalan, 2005). In addition; plant products such as flax seeds and peanuts contain cholesterol-like compounds called phytosterols, which are believed to compete with cholesterol for absorption in the intestines (Ostlund et al., 2003).

Total fat-intake also plays a role in blood-cholesterol levels. This effect is thought to come about by changes in the quantity of cholesterol and lipoproteins that are synthesized by the body. In particular, saturated, monounsaturated and polyunsaturated fats have been shown to increase HDL-based cholesterol levels, with saturated fats also

increasing LDL-based cholesterol levels (Mensink and Katan, 1992). Trans fats have been shown to reduce levels of HDL whilst increasing levels of LDL (Ascherio and Willett, 1997).

4.4 Biosynthesis

Konrad Bloch (Westheimer and Lipscomb, 2002) and Feodor Lynen (Krebs and Decker, 1982) shared the Nobel Prize in Physiology or Medicine in 1964 for their discoveries concerning the mechanism and regulation of cholesterol and fatty acid metabolism. All animal cells manufacture cholesterol with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal gland and reproductive organs (Rhodes et al., 1995).

4.5 Plasma transport and regulation of absorption

Cholesterol is only slightly soluble in water; it can dissolve and travel in the water-based bloodstream at exceedingly small concentrations. Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins, complex discoidal particles that have an exterior composed of amphiphilic proteins and lipids whose outward-facing surfaces are water-soluble and inward-facing surfaces are lipid-soluble; triglycerides and cholesterol esters are carried internally. Phospholipids and cholesterol, being amphipathic, are transported in the surface monolayer of the lipoprotein particle (Tymoczko et al., 2002; Weingärtner et al., 2010).

In addition to provide a soluble means for transporting cholesterol through the blood, lipoproteins have cell-targeting signals that direct the lipids they carry to certain tissues. For this reason, there are several types of lipoproteins within blood called, in order of increasing density, chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Gordon et al., 1989; Lewis and Rader, 2005).

4.6 Clinical significance

4.6.1 Hypercholesterolemia is strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the HDL particles, LDL particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL, which can remove cholesterol from cells and atheroma, offer protection and are sometimes referred to as "good cholesterol". These balances are mostly genetically determined, but can be changed by body build, medications, food choices and other factors (Durrington, 2003; Brunzell et al., 2008).

4.6.2 Hypocholesterolemia: Abnormally low levels of cholesterol are termed hypocholesterolemia, some studies suggest a link with depression, cancer and cerebral hemorrhage. In general, the low cholesterol levels seem to be a consequence, rather than a cause, of an underlying illness (Lewington et al., 2007)

4.6.3 Cholesterol analysis by colorimetric method: Colorimetric determinations have been commonly used in the past to determine cholesterol. One of the more popular cholesterol determinations first introduced by Zlatkis et al. (1953) involves the reaction of cholesterol with a $FeCl_3$ reagent (Scott and Leo, 1989).

4.6.4 Cholesterol analysis by HPLC method: HPLC has become a very useful analytical tool in cholesterol analysis in general; its specific application to cholesterol determination has been limited because cholesterol does not have a strong absorption peak in the UV region. Cholesterol and related sterols do, however, have an unsaturation center and a functional group that absorbs in the range 203-214 nm with a maximum at 210 nm for cholesterol (Norie, 1990). HPLC offers the advantage that many separations can be achieved at ambient temperature and the separated compounds

can be recovered from the mobile phase for further analysis by complementary techniques such as GC and MS (Hoving, 1995). Many HPLC methods have been published for the determination of cholesterol esters and cholesterol in plasma (Seta et al., 1990; JIRR, 1991) and other biological sample (Norie, 1990). Beyer and Jensen used C_{18} reversed-phase HPLC to determine cholesterol in egg yolk (Scott and Leo, 1989).

5. The avian immune system

The avian immune system is divided into non-specific and specific immune mechanisms. Non-specific immune mechanisms or innate immunity response through various kinds of anatomical and physiological condition such as high body temperature, skin and mucous membrane protection, normal flora and respiratory remove of pathogens. Specific immune mechanisms or acquired immunity is divided into cellular and non-cellular components. The non-cellular component includes immunoglobulins (antibodies) and the cells which produce them. Antibodies are specific for the foreign material (antigen) to which they attach. There are three classes of antibodies that are produced in the chicken after exposure to a disease organism: IgM, IgG and IgA. IgG is the important protective antibody in the chicken and is measured by most serological test systems. The cells which produce antibodies are called B-lymphocytes. These cells are produced in the embryonic liver, yolk sac and bone marrow. The cells move to the bursa of Fabricius (BF) after 15 days incubation through 10 weeks of age (Chansiripornchai and Sasipreeyajan, 2009). Destruction of the BF at a young age by Gumboro disease or Marek's disease prevents programming of B-cells.

When a disease organism enters the body, it is engulfed by a phagocytic-type cell, the macrophage. The macrophage transports the disease organism and exposes it to the B-lymphocytes. The B-cells respond by producing antibodies after day 5 following exposure. If the chicken is exposed a second time to the same disease, the response is quicker and a much higher level of antibody production occurs. Antibodies do not have the capability to kill viruses or bacteria directly.

Antibodies perform their function by attaching to disease organisms and blocking their receptors.

The T-lymphocytes include a more heterogeneous population than the B-cells. Some T-cells act by producing lymphokines; others directly destroy disease organisms; some T-cells act to enhance the response of B-cells, macrophages, or other T-cells (helpers); and others inhibit the activity of these cells (suppressors). The cellular system is identified when it is shown that chickens with damaged BF could still respond to and eliminate many disease organisms.

A chicken may become immune to a disease organism by producing antibodies itself or by obtaining antibodies from another animal. When the chicken produces its own antibodies following exposure to a foreign material, the process is called active immunity. This occurs after the bird is exposed to a vaccine or a field disease challenge. Active immunity is harmed by anything which damages the cellular or humoral immune systems.

When the chick receives pre-made antibodies from the hen through the egg, this is termed passive immunity. These antibodies are not produced by the chick. Maternal antibodies are present in the yolk, albumin and fluids of the egg. If the hen has a high antibody titer level to a disease, the chick should also be immune for several weeks. However, since the immune system of the chick is not stimulated, there will be no antibodies produced by the chick and no memory cells. The flock manager must be aware of the maternal antibody levels in the chicks to schedule vaccinations. If chickens are vaccinated when maternal antibody titer levels are elevated, the vaccine may be buffered excessively resulting in a reduced response. Conversely, if vaccinations are delayed and maternal titer levels are low, a severe vaccine reaction may result.

6. *Salmonella*

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 μm, lengths from 2 to 5 μm and flagella which grade in all directions (i.e. peritrichous). They

are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes. Most species produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as TSI. Most isolates exist in two phases: a motile phase I and a nonmotile phase II. Cultures that are nonmotile upon primary culture may be switched to the motile phase using a Cragie tube. Salmonella is closely related to the Escherichia genus and are found worldwide in cold- blooded and warm-blooded animals (including humans) and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever and foodborne illness (Gast, 1997).

7. Infectious bursal disease

Infectious bursal disease (IBD) or Gumboro disease has been considered as one of the important viral diseases threatening the poultry industry worldwide. It is an acute, highly contagious viral infection of young chickens and has lymphoid tissue as its primary target with special predilection of bursa of fabricius. The disease is characterized by sudden onset, short course, extensive destruction of lymphocytes and profuse watery diarrhoea, followed by death or rapid recovery (Lukert and Saif, 2003). The economic importance of the disease is manifested in two ways; firstly some virus strains may cause up to 20-30% mortality in three weeks and older chickens. The second and most important manifestation is severe, prolonged immunosuppression of chickens infected at an early age (van den Berg, 2000; Chansiripornchai and Sasipreeyajan, 2009).

The infectious bursal disease virus (IBDV) is a dsRNA virus, which is a member of genus Avibirnavirus of family birnaviridae. This virus exists in two antigenically distinct serotypes i.e. I and II. The serotype I strains are pathogenic to chickens and vary in their virulence, whereas serotype II strains are present only in turkeys (Lukert and Saif, 2003).

Due to its worldwide occurrence, IBD drew the attention of research workers for an effective control. There is no effective control of IBD except vaccination

and biosecurity. The diagnosis of any disease is the first and foremost requirement for its effective control. Routine diagnosis of IBD has been conducted by using immunodiffusion, immunoflourescence, virus neutralization, enzyme linked immunosorbent assay, electron microscopy and monoclonal antibodies (Lukert and Saif, 2003). Most of these tests require known serum against this disease. The specific hyperimmune serum is also very helpful in the differential diagnosis of disease during an outbreak. Locally, the diagnostic serum is not being produced commercially and the imported one is very expensive.

The cornerstone of many infectious disease control programs is induction of specific immunity by vaccination with either live or inactivated microorganisms or their products. An effective vaccination procedure allows the development of a state of immunologic memory in an animal for an antigen, which leads to a rapid and robust immunological response in the animal, upon subsequent contact with the antigen. Some antigens, however, are only weakly immunogenic. Such antigens may be unable to induce an immune response sufficient to provide an effective protection for the animal upon subsequent challenge, or may require administration with additional agents that boost immunogenicity to provide effective protection.

8. Newcastle disease

Newcastle disease (ND), caused by ND virus (NDV) which is an Avulavirus (Fauquet et al., 2005) is one of the most important disease encountered in the poultry industry. The first reported ND outbreak occurred in 1926 in Java (Indonesia) (Kraneveld, 1926). ND infection takes place through virus inhalation or ingestion and its spread from one bird to another depends on the availability of the virus in its virulent infectious form (Whiteman and Bickford, 1983) and its short incubation period of 5–6 days (Chansiripornchai and Sasipreeyajan, 2006). The disease normally affects the respiratory, gastrointestinal and nervous systems. Clinical signs associated with ND often begin with listlessness, increased respiration rate and weakness followed later by prostration and death. Morbidity and mortality rates of infected birds vary from 1–100%

(Alexander, 1988) with the former reaching up to 100% and with the later escalating to 50% in adult birds and 90% in young chickens.

A tentative diagnosis of NDV may be made on the basis of history, clinical signs and gross lesions but because of similarities to other diseases such as fowl cholera and highly pathogenic avian influenza, confirmation requires virus isolation and identification (Alexander, 1997).

Vaccination with viable or inactivated oil emulsion vaccines, or both, can markedly reduce the losses from NDV in poultry flocks. If eradication of the virus is not the goal of the control program, vaccines can be used to lessen the impact of the disease. Their use, however, can make the complete eradication of the virus much more problematic by increasing the difficulty of identifying infected flocks. There is little doubt, however, that vaccination makes the flock more refractive to infection when exposed and reduces the quantity of virus shed by infected flocks (Alexander, 1997).

Many ND vaccines deteriorate after storage for one or two hours at room temperature. This makes them unsuitable for use in villages where the vaccine may need to be transported for hours or in some cases days at ambient temperature. The ND vaccine is more robust and is known as a thermostable vaccine. Thermostable vaccines still require long-term storage in the refrigerator. However during transportation of the vaccine to the field, the vaccine will not deteriorate as quickly as the traditional vaccines. Evaporative cooling provided by wrapping the vaccine in a damp cloth will be adequate for maintaining the viability of the vaccine during transportation to remote villages. However if it is stored in direct sunlight or allowed to reach high temperatures (above 37^{o}C) for more than a few hours it too will deteriorate and be unsuitable for use as a vaccine (Alexander, 1997).

8.1 Immunity to Newcastle disease virus

Chickens that survive from infection with virulent NDV develop a long lasting immunity to further infection with NDV. The basis of this immunity is circulating

antibodies, secreted antibody producing mucosal immunity and cell mediated immunity. (Spradbrow,1987)

NDV of low virulence induces similar immune responses without causing severe disease. This is the basis of vaccination.

8.2 Live vaccines

These vaccines are made with virus that is alive and able to infect cells. Strains of virus of low or moderate virulence are used. They mimic natural infection and induce all three immune responses (Bennejean et al., 1978).

8.3 Killed vaccines

The ability of the virus to infect cells has been destroyed by treatment with a chemical, radiation or heat. These vaccines invoke only a circulating antibody response (Allan and Gough, 1974).

8.4 Some vaccine strains of Newcastle disease virus

Strains of NDV have been broadly classified into three pathotypes based on their virulence in chickens. Lentogenic strains are the least virulent, mesogenic strains are moderately virulent and velogenic strains are the most virulent. Most strains cluster toward the two extremes of virulence and are either lentogenic or velogenic. Velogenic viruses can be subdivided into a neurotropic form, which is typically associated with respiratory and neurologic signs and a viscerotropic form with hemorrhagic intestinal lesions (Spradbrow, 1987).

CHAPTER III

MATERIALS AND METHODS

1. Materials

1.1 Chemicals

Chemical	Grade	Supplier/ Manufacture
Acetonitrile	HPLC reagent grade	Labscan, Ireland
Cholesterol	HPLC reagent grade	Sigma Chemical Co, USA
Citric acid	Analytical reagent grade	Fisher Scientific, UK
Commercial broiler diet		Betagro, Thailand
Dextrose	Analytical reagent	E. Merck, Germany.
Dichromethane	HPLC reagent grade	Asia Pacific Specialty Chemical Limited, Australia
Disodium hydrogen phosphate anhydrous (Na_2HPO_4)		Fluka, Switzerland
Ethanol	Analytical reagent grade	The Government Pharmaceutical Organization, Thailand
Ethylenediaminetetraacetic acid (EDTA)	Analytical reagent grade	E. Merck, Germany

Chemical	Grade	Supplier/ Manufacture
Hexane	HPLC reagent grade	Mallinckrodt Chemical Co, Paris, France
Hydrochloric acid	Analytical reagent grade	Mallinckrodt Chemical Co, Paris, France
Chick N-K, Kimber strain		Fort Dodge, Brazil
Nobilis ND broiler, ND virus Clone 30		Intervet, Holland
IBD-blen, Delaware 2512 mod. strain		Merial, USA
Newcastle Disease vaccine (B1 Type, B1 strain)		Merial, USA
Methanol	HPLC reagent grade	Fisher Scientific, UK
Mineral oil		Sigma Chemical Co, USA
Plate Count agar	Analytical reagent grade	E. Merck, Germany
Potassium hydrogenphopate (KH_2PO_4)	Analytical reagent grade	E. Merck, Germany
Potassium hydroxide	Analytical reagent grade	E. Merck, Germany
Propanol-2-ol	HPLC reagent grade	Asia Pacific Specialty Chemical Limited, Australia
Sodium chloride	Analytical reagent grade	E. Merck, Germany

Chemical	Grade	Supplier/ Manufacture
Sodium dihydrogen phosphate	Analytical reagent grade	E. Merck, Germany
Sodium hexametaphosphate	Analytical reagent grade	Carlo. Erba, Germany
Sodium citrate	Analytical reagent grade	E. Merck, Germany
Sodium hydrogencarbonate	Analytical reagent grade	E. Merck, Germany
Span80		Sigma Chemical Co, USA
Tween80		Sigma Chemical Co, USA
Xylose lysine tergitol 4 agar		E. Merck, Germany

1.2 Plant material

Fresh fruit-rinds of durian, *Durio zibethinus* Murr. "Montong", was collected during the durian season from Rayong province, the rinds were cleaned, ground and dried. Dried durian rinds were kept in dried and cold place until used.

2. Equipments

- Analytical balance (Mettler Toledo, PL602-5, Switzerland)
- Autoclave HA-3D (Hirayama Manufacturing Corp., Japan)
- Blender (Moulinex 327, Spain)
- Column type symmetry C_{18} (3.5 x 150 mm, 5 μ) (Waters, USA)
- Column type μ porasil (3.9 mm. X 30 cm) (Waters, USA)
- Circuit breaker (Nisshin Denko Co. Ltd., Japan)

- ELISA test kits (Synbiotic Corp., USA)
- Hot air oven (Mammert, Becthai Co., Ltd., Thailand)
- HPLC pump (LC-10AD Liquid Chromatograph, Shimadzu Corp., Japan)
- HPLC detector (SPD-10A UV-VIS Detector, Shimadzu Corp., Japan)
- Incubator model 6 (Thelco, USA)
- Magnetic stirrer type KMO 2 electronic (Janke & Kunkel, Kika-Werk)
- Membrane filter sterile, pore size 0.45 μm (Whatman, England)
- Pan coating machine (Yinrich, P1250, China)
- Pipetman (Gilson, France)
- pH meter type MP 230 (Mettler Toledo, Switzerland)
- Refrigerator (Sharp TH-8903, Thailand)
- Rotary evaporator (Buchi R-200, Switzerland)
- Separatory funnel (Kimax, USA)
- Viscometer (Brookfield, model LVDV-I+, Brookfield Engineering Laboratories INC., USA)
- Vortex type retsch mixer
- Water bath (Edelstahl, Rostfrei)

3. Experimental animals

Unvaccinated female Cobb 500 broiler chicks obtained from a commercial hatchery (Krungthai, Thailand), The chickens were fed *ad libitum*, chickens were raised in a cage on an elevated platform at room temperature. At the onset of the experiment, no statistically significant difference in the average weight between the control and the experimental groups were observed. Guidelines and legislative regulations on the use of animals for scientific purposes by Chulalongkorn University, Bangkok, Thailand were followed as is protocol review No. 0831048.

4. Preparation of polysaccharide gel (PG) feed additive

Extraction of Polysaccharide del (PG) has been previously reported (Pongsamart and Panmaung, 1998; Hokputsa et al., 2004). Briefly, the PG was extracted from the dried fruit-rind of durian with boiling water and filtered. The PG water extract was concentrated under reduced pressure by using rotary evaporator and precipitated in acidified aqueous ethanol, filtered, washed with 70% ethanol, dried at 50°C and ground. The pale beige color powder of durian polysaccharide called "PG" was used in the experiment.

5. Experiments

Part 1: Effects of feed additive containing polysaccharide gel from fruit-rind of *Durio zibethinus* Murr. on immune response and cholesterol levels in plasma and muscle of chickens.

1. Preparation of polysaccharide gel feed-additive diet

Ten grams of the PG were dispersed in water and then spray-coated onto 100 g the granules of commercially available broiler diet, in a pan coating machine to make the stock feed additive diet containing 10 g% PG.

commercial feed containing 1.0 g% : Mixed 10 g of stock feed additive diet containing 10 g% PG with 90 g of commercial feed.

commercial feed containing 2.0 g% : Mixed 20 g of stock feed additive diet containing 10 g% PG with 80 g of commercial feed.

commercial feed containing 3.0 g% : Mixed 10 g of stock feed additive diet containing 30 g% PG with 70 g of commercial feed.

The broiler diet was analyzed for nutritional components before used for feeding the experimental chickens.

2. Experimental designs

2.1 Experimental broilers

Eighty broilers were randomly allocated into four groups. Each group of chickens were divided into 2 replicates in each group.

Group 1: Two replicates of negative control group, broilers were fed with commercial feed without PG.

Group 2: Two replicates of experimental group, broilers were fed commercial feed containing 1.0 g% PG.

Group 3 : Two replicates of experimental group, broilers were fed commercial feed containing 2.0 g% PG.

Group 4 : Two replicates of experimental group, broilers were fed commercial feed containing 3.0 g% PG.

Each group was fed *ad libitum* for 6 weeks, broilers were raised in a cage on an elevated platform at room temperature. Chicken feed was supplied everyday, 1 kg/day/replicate, chicken weights and diet consumption in each group was record every week for 6 weeks.

2.2 Broiler vaccination program

At one-day-old, live ND vaccine (Newcastle disease vaccine, B1 Type, B1 strain, Merial, USA) were administered by eye drop to every chicken in each groups. At 7-day-old, all of the chickens were subcutaneously injected with inactivated oil adjuvant ND vaccine (Nobilis ND broiler, ND virus Clone 30, Intervet, Holland). At 14-day-old, all

chickens were orally given with IBD vaccine (IBD-blen, Delaware 2512 mod. strain, Merial, USA) as the following table .

Age	Vaccines	Route
1	Newcastle disease vaccine, B1 Type, B1 strain	eye drop
7	Nobilis ND broiler, ND virus Clone 30	subcutaneous injection
14	IBD-blen, Delaware 2512 mod. strain	oral drop

All chicks were bled at one-day-old, before starting the experiment. The broilers in each group were bled at 7, 14, 21, 28, 35 and 42 day-old to measure H:L ratio, hemagglutination-inhibition (HI) titers for Newcastle disease and ELISA for infectious bursal disease. At 42-day-old the pectoral muscle of 6 chickens in each group was randomly selected for cholesterol analysis by using HPLC method. Plasma of chicken at one and 42-day-old were collected to analyze cholesterol level in plasma. Chickens weight and the amount of feed intake were recorded for the calculation of body weight gain and feed conversion ratio (FCR). The feces were collected to determine the colony count of total bacteria and the colony count the bacterium *Salmonella*. Body weight and feed consumption were record every week for 6 week.

3. Serum samples

Blood samples were collected from the wing vein at 7, 14, 21, 28, 35 and 42 day-old and then centrifuged at 1,000xg for 15 minutes. Sera were separated, frozen and stored at -20^{o}C for determination of the hemagglutination-Inhibition test (HI) and ELISA test.

4. Plasma samples

At one-day-old and 42-day-old, chickens' plasma was collected using EDTA as an anticoagulant. The samples were centrifuged at 1,000 g for 15 minutes, plasma was collected and stored at -20°C for the plasma cholesterol determination.

5. Heterophil:Lymphocyte (H:L ratio)

Bloods were collected at 7, 14, 21, 28, 35 and 42 day-old using EDTA as anticoagulant and carried out to measure the ratio of heterophil:lymphocytes (H:L). The bloods were smeared and stained with Wright-Giemsa (Lucas and Jamroz, 1961), approximately 2 to 4 hours after preparation by methyl alcohol fixation. One hundred leukocytes, including granular (heterophils, eosinophils and basophils) and nongranular (lymphocytes and monocytes), were counted under light microscope and the heterophil to lymphocyte ratio was calculated. The bloods were analyzed by Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University.

6. Hemagglutination-inhibition and ELISA test

The hemagglutination-inhibition (HI) titers of ND were tested in a U-shaped, 96-well, microtiter plate which was carried out as the method modified by Alexander (2000). In brief, a dilution series of sera was incubated with 4 hemagglutination unit (HAU) of ND virus, La Sota strain at room temperature for 30 minutes. The HAU was titrated before each assay. Thereafter, chicken erythrocytes were added and agglutination was monitored after incubation at room temperature for 45 minutes. The HI titer was defined as the reciprocal of the highest serum dilution completely inhibiting agglutination.

The antibody titers of IBD were determined using ELISA test kit. Briefly, ELISA plates are coated with IBDV specific antibodies. Samples serum diluted 1/10 to 1/25

(w/v) in a dilution buffer are incubated in the coated wells. Unbound antigens are discarded at the end of the incubation period by washing with a washing buffer. The captured antigens are then revealed, as in an indirect ELISA, with a detection antibody, followed by an enzyme conjugate that binds to the detection antibody only followed by the enzyme substrate. Finally, optical densities, which parallel the amount of captured IBDV antigens, are read with an ELISA reader (Eterradossin et al., 1997)

7. **Determination of total bacteria count and number of *Salmonella* colonies.**

7.1 Preparation of samples. A sample collection technique was modified from the method as described by Corrier et al. (1994). The samples of chicken feces were collected from 5 spots in a fecal tray; 4 spots at the corner and 1 point at the center of the tray and then pooled into 1 sample. Each collected sample was further tested for *Salmonella* colonies and total bacteria count. One gram fecal samples were taken and mixed with 9 ml of buffered peptone water (BPW), pH 7.5. The fecal suspension samples were used for the determination of the number of total bacteria count and *Salmonella* colonies.

7.2 Total plate count (TPC). The fecal suspended samples were diluted by ten fold serial dilution with buffered peptone water from 10^{-2} to 10^{-13} dilution. Plate count agar medium was melted and poured into a petri dish, cooled until solidified at room temperature and then a 0.1 ml sample was pipetted from the 10^{-2} to 10^{-13} dilution into each petri dish, spread plate was performed and incubated at 35°C for 24 hours. A plate that contained total colonies of bacteria between 30 and 300 colonies was used for bacteria plate counting (ISO, 2002).

7.3 Salmonella colonies count. Fecal samples were taken for counting the number of colonies of *Salmonella*. The method was carried out as follows: 1 ml of the fecal suspension of each sample, obtained by mixing the fecal sample with buffered peptone

water (1:9 w/v), was transferred into 9 ml of buffered peptone water; this suspension mixture was diluted to 10^{-1}. A sample of 0.1 ml of the diluted solution was transferred to a plate of xylose lysine tergitol 4 agar and incubated at 37°C for 24 h. Then the black colonies of *Salmonella* were counted (Xiong et al., 1998).

8. Determination of cholesterol

8.1 Extraction of cholesterol from plasma. Cholesterol was extracted from 0.2 ml of chicken plasma which was collected at one-day and 6 weeks of age with 0.5 ml cold methanol and 2.5 ml hexane. The samples were vortex and then centrifuged: 550xg at 4°C for 5 minutes, the hexane layer was removed and dried under N_2 and re-dissolved in mobile phase which composed of acetonitrile:isopropanol (7:3, v/v) 1 ml and transferred to a HPLC vial and then 15 μl sample was analyzed with HPLC instrument by using column C_{18} (3.5 x 150 mm, 5 μ).

8.2 Extraction of cholesterol from breast muscle. Twenty four chickens from the 4 experimental groups were randomly euthanized. The breast muscle of each chicken was collected and cholesterol was extracted by the method of Folch et al. (1957). Briefly, one hundred grams of breast muscle were minced, 0.5 g aliquot of the homogenate was heat with 3 ml, 95% ethanol and 2 ml, 50% of potassium hydroxide at 60°C for 10 minutes. Later, the samples were extracted with 5 ml of hexane and 3 ml of distilled water allowed to stand until the two layers were separated and the cholesterol in a hexane layer was dried with blowing N_2 gas and diluted with 1 ml of acetonitrile:isopropanol (7:3, v/v) and 15 μl sample was analyzed with HPLC method.

8.3 Analysis of cholesterol. The cholesterol content was assayed using HPLC technique (Araki et al., 1990; Seta et al., 1990), a column symmetry C_{18} (3.9X150 mm, 5 μm) was used and each of 15 μl sample was injected into the column. The mobile phase was acetonitrile:2-propanol (7:3) which was previously filtered through a 0.45 μm membrane

filter before used. The column was eluted at a flow rate of 1.5 ml/minute at ambient temperature and the UV detector was monitored at 210 nm. Each peak of cholesterol content in a sample was calculated by using peak area of sample and cholesterol standard and 0.504 mg of 6-ketocholestanol was used as an internal standard.

8.4 HPLC technique for cholesterol analysis

Chromatographic condition :

Reverse phase HPLC column was used: Symmetry C_{18} (3.9X150 mm, 5 μm). Mobile phase was a mixture of acetonitrile:2-propanol (7:3), which was previously filtered through a 0.45 μm membrane filter before used. The column was eluted at a flow rate of 1.5 ml/minute at ambient temperature and monitored at 210 nm UV detector.

Procedure analysis :

Cholesterol and internal standard were dissolved in methanol to prepare stock standard solutions. The standard stock solutions were stored at 4^{o}C.

Calculation :

Identification and quantification of the eluted cholesterol standards and samples were examined by comparing its retention time and integration the standard curve of the average peak area of cholesterol.

Part 2: Effects of polysaccharide gel (PG) from fruit-rind of *Durio zibethinus* Murr. as an vaccine adjuvant.

1. **Preparation inactivated Newcastle disease vaccines.**

1.1 Virus preparation

1.1.1 The local strain of very virulent ND virus (vvNDV), NDV-CU-1, was used for this preparation.

1.1.2 The solution was filtered through a 0.45 micron nitrocellulose membrane.

1.1.3 A volume of 0.2 ml viral solution was propagated in 9-day-old embryonated eggs at allantoic cavity.

1.1.4 The embryonated egg was incubated in the incubator at 35°C, 55-66% humidity, for 48 hours.

1.1.5 The allantoic fluid (AF) from each egg was centriguged at 1,000xg for 15 minutes and the supernatant was collected.

1.1.6 The supernatant was isolated from 10 ml of AF. The concentration test of virus was evaluated by Hemagglutination (HI) test and the 50 percent Embryo Lethal Dose or ELD_{50} was determined.

The intracerebral pathogenicity index (ICPI) of the local NDV strain was 1.8 (Chansiripornchai and Sasipreeyajan, 2006). The concentration of $10^{5.7}$ ELD_{50}/dose was used as a stock virus for vaccine preparation. The concentration of virus vaccine was identified by hemagglutination (HA) test (OIE, 2004). The NDV crude antigen for producing vaccines in these studies had HA titer of 256 HAU/50 μl. The virus was inactivated by treatment with 0.05% formaldehyde as described by OIE (2004) and inoculated into the chicken embryonic eggs for 2 passages intended to assure the inactivation efficacy.

1.2 Preparation of inactivated vaccines

1.2.1 The formulation of vaccine was prepared. An aqueous phase of vaccines emulsion consisted of NDV. The ratio of the aqueous phase to the oil phase of the vaccine emulsion was 1:4. The water in oil emulsion of vaccine was prepared as the following:

Preparation of PG dispersion : Preparation of 3 g% PG as a stock solution was made; 3 g PG was dispersed in 100 ml distilled water. The PG dispersion was used for the preparation inactivated vaccine that contained 0.5, 1.0 and 1.5 g% PG.

Oil Phase : A volume 187.5 ml of mineral oil was mixed with 12.5 ml of Span80 in Erlenmeyer flask and mixed by using a magnetic stirrer to make volume of 200 ml the oil phase.

Aqueous phase

Aqueous phase formula 1 (0% PG) : Mixed 2.51 ml of stock virus solution with 1.6 ml of Tween80 and adjust to the volume of 50 ml with distilled water in a volumetric flask and mixed by using a magnetic stirrer.

Aqueous phase formula 2 (0.5% PG) : stock virus solution 2.51 ml, Tween80 1.6 ml, 8.33 ml of 3g% PG and adjust the volume to 50 ml with distilled water in Volumetric flask and mixed by using a magnetic stirrer.

Aqueous phase formula 3 (1.0% PG) : stock virus solution 2.51 ml, Tween80 1.6 ml, 16.67 ml of 3g% PG and adjust the volume to 50 ml with distilled water ml in Volumetric flask and mixed by using a magnetic stirrer.

Aqueous phase formula 4 (1.5% PG) : stock virus solution 2.506 ml, Tween80 1.6 ml, 25 ml of 3g% PG and adjust the volume to 50 ml with distilled water and mixed.

1.2.2 Gently pour the aqueous phase into the oil phase while continuing homogenized by using a homogenizer for 5 minutes. The prepared vaccines were kept at 4°C until used.

2. The viscosity, stability and tissue reaction tests

2.1 The viscosity of the vaccines was examined by using Viscometer.

2.2 The stability of the vaccine was tested by observing the vaccine preparation at 4°C and 25°C for 4 weeks in the tightly screw-capped tubes until the emulsion vaccine has clearly separated to the water phase and oil phase (Stone, 1997).

2.3 The tissue reaction was examined by observing subcutaneously of 3 chickens per treatment at the injection sites. The lesion has been scored in 3 levels (Stone et al., 1978).

- The mild level meant pale muscles around 1 cm and no vaccine left at the injection site.

- The intermediate level meant pale to red muscles around 1-3 cm in the superficial muscles at the injection site and the small droplets of vaccine left.

- The severe level meant red and inflammable muscles around 3-4 cm in the superficial and deep muscles at the injection sites and the vaccine was observed when the muscle was dissected

3. Experimental designs

One hundred and sixty eight broilers were randomly allocated into 7 groups. Chickens were divided into 3 replicates of 8 chicks.

Group 1: negative control group, unvaccinated, unchallenged.

Group 2: positive control group, unvaccinated, challenged with local strain of vvNDV.

Group 3 : at 21-day-old, broiler chickens were received inactivated NDV vaccine that without PG (0% PG) in the dose of 0.5 ml/ chick s/c route at the nape of the neck.

Group 4 : at 21-day-old, broiler chickens were received inactivated NDV vaccine that with PG (0.5% PG) in the dose of 0.5 ml/ chick s/c route at the nape of the neck.

Group 5 : at 21-day-old, broiler chickens were received inactivated NDV vaccine that with PG (1.0% PG) in the dose of 0.5 ml/ chick s/c route at the nape of the neck.

Group 6 : at 21-day-old,, broiler chickens were received inactivated NDV vaccine that with PG (1.5% PG) in the dose of 0.5 ml/ chick s/c route at the nape of the neck.

Group 7 : at 21-day-old, broiler chickens were received a commercial inactivated vaccine in one dose/chick following the company suggestions.

The feed intake *ad libitum* was carried out for 55 days. Body weight and feed consumption was recorded for calculation of the feed conversion ratio (FCR).

4. Disease resistance test

At 42-day-old, the chickens were challenged orally with local strain of vvNDV (Chansiripornchai and Sasipreeyajan, 2006.) at the concentration of $10^{5.7}$ ELD_{50}/dose according to British Pharmacopoeia (Veterinary) (1998). Morbidity and mortality rates were observed for 13 days.

5. Sample collection

Blood was collected at 1, 7, 14, 21, 28, 35, 42 and 55 day-old from the wing vein and tested hemagglutination-inhibition (HI) titers for ND. Chickens at 1 and 55-day-old in each group were weighed and the amount of feed intake was recorded and used for the calculation of the feed conversion ratio (FCR). Body weight and feed consumption in each week was recorded for 55 days.

Serology

The HI titers of ND in the chicks were determined in an U-shaped, 96 well, microtiter plate as the following modified method as described by OIE (2004). In brief, the two fold serial dilutions of serum were incubated with four hemagglutinating units (HAU) of ND virus La Sota Strain at room temperature for 30 minutes. The HAU was titrated before each assay.

Thereafter, chicken erythrocytes (1% v/v in PBS) were added and agglutination was monitored after incubation at room temperature for 45 minutes. The HI titer was defined as the reciprocal of the highest serum dilution that completely inhibited agglutination.

Statistic analysis

Data were analyzed using One-way ANOVA followed by Post Hoc Multiple Comparisons. In all analyses a value of $P<0.05$ was considered significant. All statistical analyses were performed by using SPSS 13.0 for WINDOWS (SPSS Inc, Chicago).

CHAPTER IV

RESULTS

Part 1: Effects of feed additive containing polysaccharide gel (PG) from fruit-rind of durian, *Durio zibethinus* Murr. on immune response and cholesterol levels in plasma and muscle of chickens.

1. Impact of PG on feed conversion ratio.

Commercially available chicken diet composed of 20 g protein, 56.56 g carbohydrate, 5.94 g lipid, 4.66 g ash and 12.84 g moisture in 100 g diet as shown in the Table 4-1. The PG additive diets were prepared by coating the granules of chicken diet with PG aqueous dispersion to prepare PG additive diet containing 1, 2 and 3 g PG, respectively, in 100 g of the chicken diet.

The results indicated that PG in the different ratio (1-3%) in a feed additive diet did not affect the chicken's weight as well as total feed intake. The results revealed no significant difference between the groups of control and treatment fed with the commercial feed without PG (control) and the groups fed with the commercial feed coated with PG 1, 2 and 3 g/100 g (Table 4-2). The FCR values were not significantly different. However, the groups fed with the commercial feed with PG additive diet tended to show lower FCR compared with the control group fed a diet without PG.

Table 4-1: The nutritional content of commercial feed of chicken.

commercial feed composition	g/100 g feed
Protein	20.00
carbohydrate	56.56
lipid	5.94
ash	4.66
moisture	12.84

Table 4-2: The effect of PG on body weight gain and FCR in chickens (mean±SE).n =20

Groups	Weight of chickens after treatment (g) (mean±SE)	Weight gain of chickens (g) (mean±SE)	Feed intake (g) (average wt.)	FCR (feed intake(g)/g wt. gain)
1(0% PG)	1899.44±2.21	1856.44±2.21	4024.40	2.17
2(1% PG)	1916.67±10.29	1873.67±10.29	3890.29	2.08
3(2% PG)	1972.63±8.71	1929.63±8.71	3714.83	1.93
4(3% PG)	1961.58±5.63	1918.58±5.63	3922.86	2.04

No significant differences ($P<0.05$) between groups fed with commercial feed without PG and groups fed with commercial feed coated with PG.

2. The effect of PG on hemagglutination-inhibition (HI) titers against Newcastle disease (ND) and ELISA titers against infectious bursal disease (IBD).

At 6-week-old, HI antibody titers against ND virus (Figure 4-1) and ELISA antibody titers against IBD virus in the group 4 (Figure 4-2) were statistically significantly different (P<0.05) compared to the other groups and control. The chickens in the group 4 fed the commercial feed with PG 3 g/100 g diet revealed the highest titers against the ND and IBD antibody than the control and the other treated groups. At week 1 until week 5, the results revealed no significant difference between groups fed with the commercial feed without PG (control) and groups fed with commercial feed coated with PG 1, 2 and 3 g/100 g.

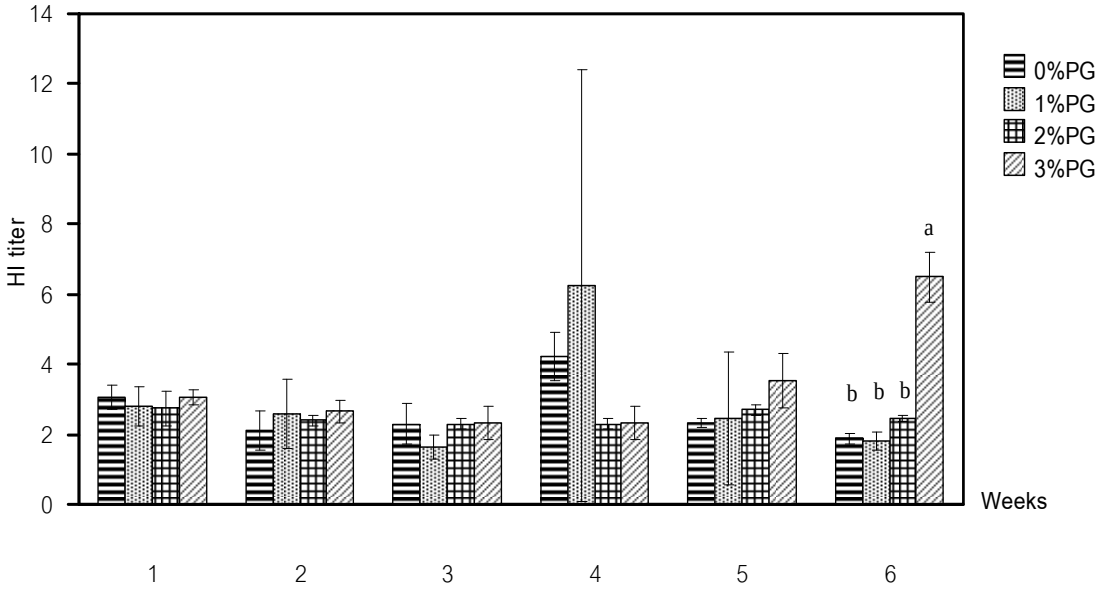


Figure 4-1: Hemagglutination-inhibition antibody titers against Newcastle disease virus were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the geometric mean titer (log2±SD) of twenty serum samples.

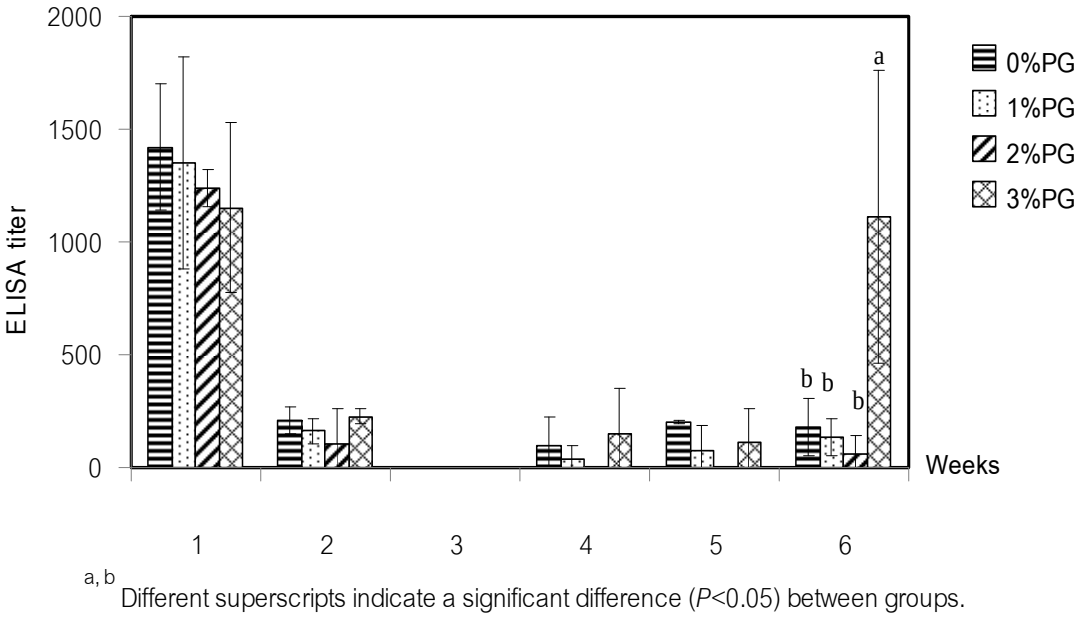


Figure 4-2: ELISA antibody titers against infectious bursal disease virus were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the arithmetic mean titer (mean±SD) of twenty serum samples.

3. The effect of PG on the ratio of Heterophil:Lymphocyte (H:L ratio).

The results of H:L ratio at the beginning until the end of the experiment, excepted for the week 5, revealed no significant difference between groups fed with the commercial feed without PG (control) and groups fed with the commercial feed coated with PG 1, 2 and 3 g/100 g (Figure 4-3). At the 5 week, the H:L ratio of chickens in each group was higher compared with those of the other weeks, however group 1 (0% g PG) of control showed the highest H:L ratio than those of the other treatment groups.

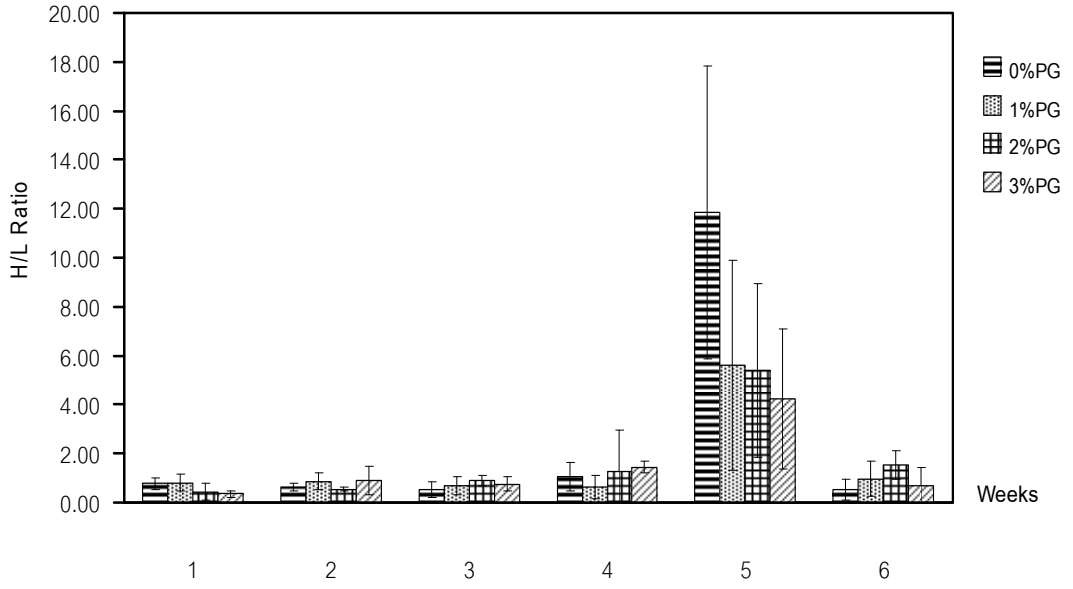


Figure 4-3: Heterophil:Lymphocyte ratio of white blood cell fraction in chickens were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the arithmetic mean (mean±SD) of twenty serum samples.

4. The effect of PG on the total bacteria count in chicken feces.

The chickens in groups 2, 3 and 4 fed with the commercial feed containing PG 1, 2 and 3 g/100 g, respectively, exhibited lower total bacteria counts than those of the chickens in group 1 (control). The PG reduced the number of total bacteria in chicken fecal samples and the increased PG in feed resulted in decreasing the total bacteria count in chicken feces. The total bacteria count at week 6 was reduced more than 90% from the bacteria count at week 1 (Table 4-3). The PG in the feed-additive diet exhibited a killing effect on the bacteria in chicken feces.

Table 4-3: Total bacteria count (10^{11} colony forming unit/ml) in fecal samples of chickens in each group/week determined weekly.

Groups	Average total bacteria count (mean±SDx10^{11} cfu/ml)					
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
1 (0% PG)	171.25±14.43^{ab}	290±7.83^{a}	36.5±7.59	301.5±5.45^{a}	160±12.25^{a}	137±19.71^{a}
2 (1% PG)	156.25±7.41^{bc}	155±26.04^{c}	45±4.97	120.5±56.04^{b}	32±15.43^{b}	26±18.24^{b}
3 (2% PG)	126.5±2.12^{c}	128±16.43^{c}	87.5±62.04	111±15.14^{b}	11±6.22^{c}	16.25±4.65^{b}
4 (3% PG)	200.75±31.91^{a}	236.5±42.61^{b}	36.75±31.08	55.75±15.84^{c}	4.75±2.22^{c}	4.5±1.73^{b}

$^{a, b, c}$ Different superscripts indicate a significant difference (P<0.05) between groups in each week.

5. The effect of PG on *Salmonella* in fecal samples of chickens.

The colonies number of *Salmonella* in chicken feces was significantly lower in groups fed with PG additive diets. The experimental groups fed commercial feed coated with PG 1, 2 and 3 g/100 g, respectively, showed a lower colony count of *Salmonella* than the group fed commercial diet without PG (Table 4-4). Diet with PG additive showed a zero colony count of *Salmonella* in group 2 and 3 in chicken feces at 5-week-old. No *Salmonella* was found in chickens fed with the PG additive diets in chickens at 6-week-old.

Table 4-4: *Salmonella* colony count (10^2 colony forming unit/ml) in fecal samples of chickens in each group/week determined weekly.

Groups	Average Salmonella suspected colony count (mean±SDx10^2 cfu/ml)					
	1 week (7 days)	2 weeks (14 days)	3 weeks (21 days)	4 weeks (28 days)	5 weeks (35 days)	6 weeks (42 days)
1 (0% PG)	272.75±25.75[a]	299±24.25[a]	350±14.00[a]	30±15	27.5±6. 50[a]	10±1.00[a]
2 (1% PG)	112.5±2.75[b]	196.25±7.50[b]	105±4.50[b]	0	0[b]	0[b]
3 (2% PG)	47.25±23.63[b]	113.5±1. 50[b]	89.75±22.25[b]	5±2.00	0[b]	0[b]
4 (3% PG)	24.75±6.25[b]	100.5±39.75[b]	16.5±6.50[b]	0	2±1.00[b]	0[b]

[a, b, c] Different superscripts indicate a significant difference (P<0.05) between groups.

6. The effect of PG on the plasma cholesterol in chickens.

The average cholesterol levels in the plasma of chickens in groups 1-4 at one-day-old and 6-week-old were shown in Figure 4-4. The results revealed no significant difference between groups fed with commercial feed without PG (control) and groups fed with commercial feed coated with PG 1, 2 and 3 g/100 g. The plasma cholesterol levels in the PG treated groups showed potentially lower cholesterol levels compared to those of the control group.

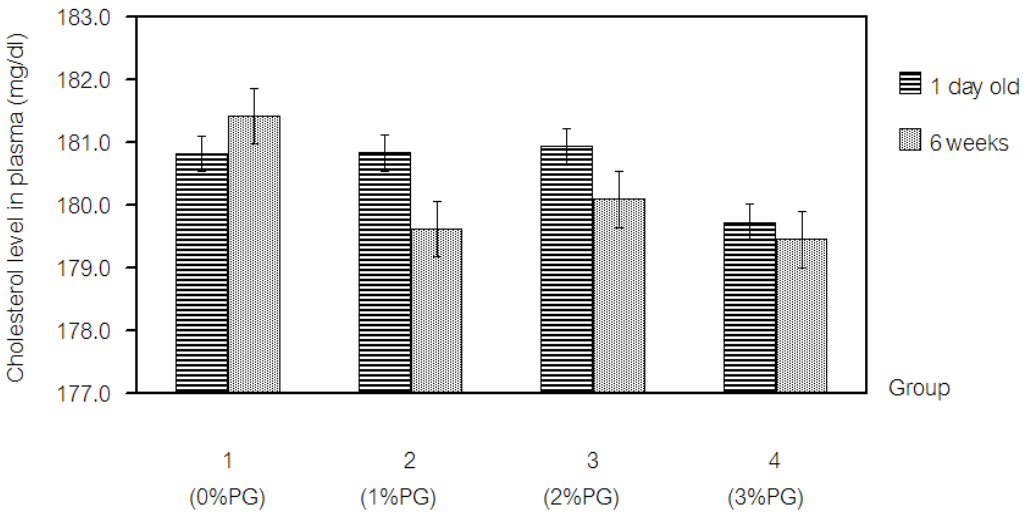


Figure 4-4: The cholesterol level in chicken plasma before and after being fed with a PG additive diet for 6 weeks. Data are mean values with SD. n=20

7. The effect of PG on cholesterol levels in chicken muscle.

The cholesterol levels in chicken muscle of each experimental group were shown in Table 4-5. The cholesterol level of chickens in group 4 (3% PG) was significantly lower than that of the chickens in group 1 (0% PG). The higher of PG in feed revealed the greater reduction of the cholesterol in chicken's muscle. Recalculation to 100% cholesterol level in the muscle of chickens in group 1 (0% PG), the cholesterol level in the muscle of chickens in group 4 was reduced to 75.78% compared with 100% cholesterol in control (Table 4-5).

Table 4-5: Cholesterol levels in 6 chicken muscle samples in each group analyzed at 6-week-old. n=6

Group	cholesterol level in chicken muscle (mg/100 g)	% of cholesterol level in chicken muscle compared to control group
1(0% PG)	61.73±1.77[a]	100.00
2(1% PG)	50.85±1.70[ab]	82.37
3(2% PG)	54.31±4.59[ab]	87.98
4(3% PG)	46.78±6.94[b]	75.78

[a, b] Different superscripts indicate a significant difference ($P<0.05$) between groups.

Part 2: Effects of polysaccharide gel (PG) from fruit-rind of *Durio zibethinus* Murr. as an vaccine adjuvant.

1. The effect of NDV vaccine on FCR.

At 55-day-old (terminated), the results indicated that PG in the different ratio in inactivated vaccine did not affect the FCR. The results in Table 4-6 revealed no significant difference between the control group, the treated group that received inactivated vaccine with 0.5 and 1.0% and 1.5% PG and commercial vaccine group.

Table 4-6: The effect of inactivated ND vaccines containing different concentrations of PG on body weight gain (mean±SE) and FCR of chickens.

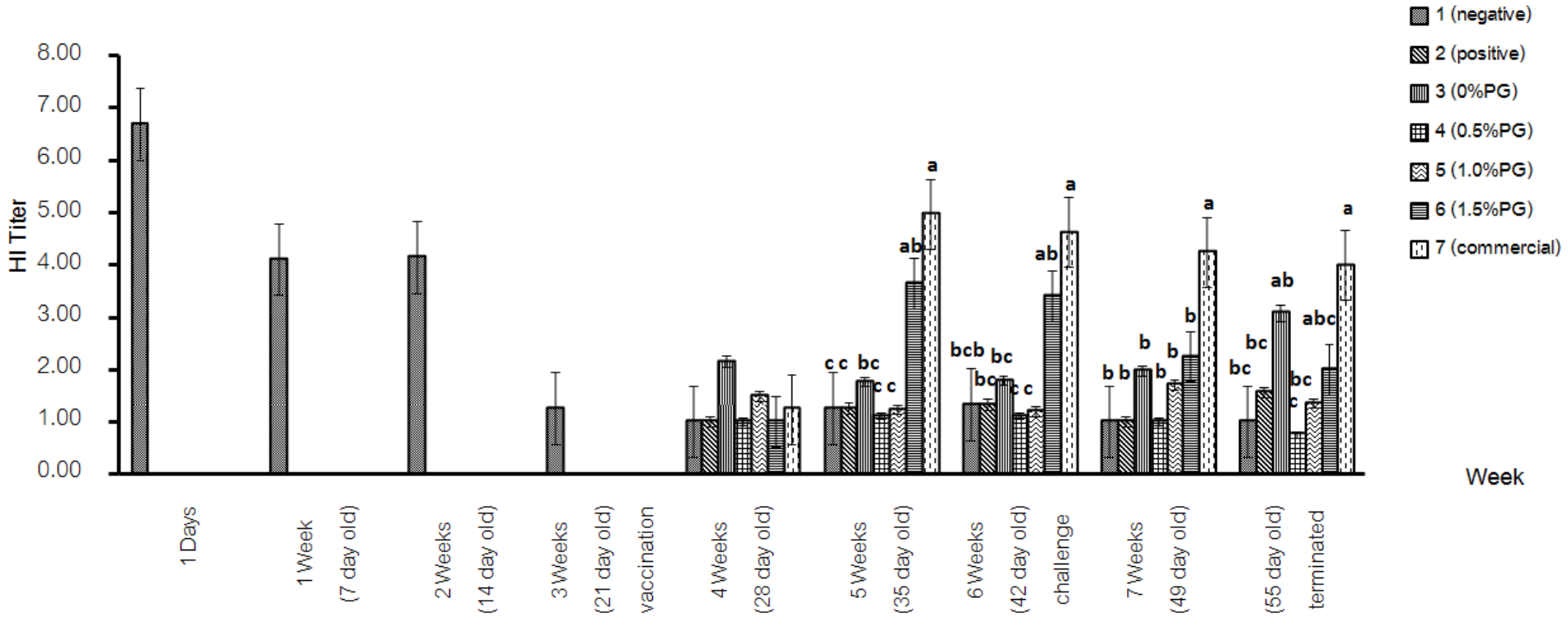
Groups	Weight gain of chickens (g) (mean±SE)	Feed intake (g) (mean±SE)	FCR (feed intake (g)/g wt. gain)
negative	2039.24±0.08[ab]	5283.69±0.01	2.59
positive	2070.78±0.05[ab]	5857.37±0.53	2.83
0% PG	2158.89±0.04[ab]	6402.76±0.62	2.97
0.5% PG	2163.81±0.02[ab]	5724.96±0.31	2.65
1.0% PG	2195.24±0.05[a]	5350.71±0.20	2.44
1.5% PG	2000.60±0.04[b]	6081.21±0.69	3.04
commercial vaccine	2131.17±0.07[ab]	5562.38±0.49	2.61

[a, b] Different superscripts indicate a significant difference (P<0.05) between groups that received inactivated ND vaccines with different concentrations of PG and control groups.

2. The effect of PG in chickens receiving different concentrations of PG containing in inactivate ND vaccines on hemagglutination-inhibition (HI) titers against Newcastle disease (ND).

After vaccine injection at week 3, the immune response was evaluated by testing HI antibody titer against ND virus at week 4, 5 and 6, respectively. The result in Figure 4-5 showed that at week 4 HI titer in negative control, positive control and treated group injected with inactivated vaccine with 0.5, 1.0 and 1.5% PG as well as commercial inactivated ND vaccine were not significant difference (P<0.05). At week 5 (14 days

after vaccination), the HI titers in negative control, positive control and treated group injected with inactivated ND vaccine contained 0.5 and 1% PG were not significant difference, but these values were lower than those groups injected with inactivated ND vaccine contained 1.5% PG and commercial inactivated ND vaccine. These 2 groups showed the highest and not significant difference in their HI titer. The HI titer were slightly decreased within 14 days of injection at week 6. All of the chickens were challenged with local strain of vvNDV at week 6. After challenge for 7 days, the HI titers in treated group injected with inactivated ND vaccine contained 1.5% PG showed sharply decreased at week 7. HI titers at week 7 showed that the HI titers in negative control, positive control and treated group injected with 0.5, 1.0 and 1.5% PG were not significant difference and these values were lower than the group injected with commercial inactivated ND vaccine. At 55-day-old the HI titer in negative control, positive control and treated group injected with 0.5 and 1.0% PG were not significant difference, but these values were lower than the group injected with commercial inactivated ND vaccine and HI titers in treated group injected with inactivated ND vaccine without PG and treated group injected with inactivated ND vaccine contained 1.5% PG were not significant difference when compared with commercial inactivated ND vaccine. Behavior of the chickens was observed after challenge, broilers in positive control were depressed than the other groups.



[a, b, c] Different superscripts indicate a significant difference ($P<0.05$) between groups that received inactivated ND vaccine added with PG and without PG.

Figure 4-5: Hemagglutination-inhibition antibody titers against Newcastle disease virus were determined weekly in chickens receiving different concentrations of PG contain in inactivate ND vaccines. Each data set represents the geometric mean titer (log2±SD) of twenty serum samples.

3. The viscosity, stability and tissue reaction tests

3.1 The viscosity tests: The vaccine formulations with PG showed lower viscosity values than the commercial vaccine and vaccine formulation without PG (P<0.05) (Table 4-7).

Table 4-7: Viscosity of the vaccine formulation.

Vaccine formula	Type	Viscosity (Centipoise, cps)			mean ± SE
		1st test	2nd test	3rd test	
1	inactivated NDV vaccine (without PG)	541.23	541.21	541.23	541.22^{e} ±0.006
2	inactivated NDV vaccine+0.5% PG	475.78	475.78	475.78	475.78^{c} ± 0.00
3	inactivated NDV vaccine+1.0% PG	475.88	475.88	475.85	475.87^{b} ±0.01
4	inactivated NDV vaccine+1.5% PG	475.68	475.68	475.68	475.68^{d} ± 0.00
5	Commercial inactivated NDV vaccine	633.45	633.45	633.45	633.45^{a} ±0.00

Note: The tests were performed at 25°C. The different superscripts in the same column were statistically significant (P<0.05).

3.2 The stability tests: Vaccine emulsion exhibited good stability after preparation and allowed to stand at 4°C, for more than 4 weeks except for the formula 4.

The 1st formulation (inactivated NDV vaccine; without PG), the 2nd formulation (inactivated NDV vaccine+0.5% PG), the 3rd formulation (inactivated NDV vaccine+1.0% PG) and the commercial inactivated NDV vaccine exhibited good stability after stand at 4°C for 4 week, while 4th formulation (inactivated NDV vaccine+1.5% PG) showed a phase separation at 16 days, after stand at 4°C (Table 4-8).

The 1st formulation (inactivated NDV vaccine; without PG), the 2nd formulation (inactivated NDV vaccine+0.5% PG), the 3rd formulation (inactivated NDV vaccine+1.0% PG) and the commercial inactivated NDV vaccine exhibited good stability after stand at 4°C for 4 week, while the 4th formulation (inactivated NDV vaccine+1.5% PG) showed a phase separation at 22 days, after stand at 25°C (Table 4-8).

Table 4-8: The stability tests.

Formulation	Type	The stability of vaccine (week)	
		4°C	25°C
1	inactivated NDV vaccine (without PG)	> 4	> 4
2	inactivated NDV vaccine+0.5% PG	> 4	> 4
3	inactivated NDV vaccine+1.0% PG	> 4	> 4
4	inactivated NDV vaccine+1.5% PG	16 days	22 days
5	Commercial inactivated NDV vaccine	> 4	> 4

3.3 Tissue reaction tests: The tissue reactions were observed for 7 days after vaccination. All vaccines revealed the most mild level of tissue reactions (Table 4-9).

Table 4-9: The tissue reaction of chickens, at 7 days after receiving each vaccine in formulation.

Formulation	Type	Tissue reaction levels at 7 day after injection		
		Mild[a]	Intermediate[b]	Severe[c]
1	inactivated NDV vaccine (without PG)	3/3	0/3	0/3
2	inactivated NDV vaccine+0.5% PG	3/3	0/3	0/3
3	inactivated NDV vaccine+1.0% PG	3/3	0/3	0/3
4	inactivated NDV vaccine+1.5% PG	3/3	0/3	0/3
5	Commercial inactivated NDV vaccine	3/3	0/3	0/3

Note: [a]pale muscles around 1 cm and no vaccine left at the injection site.

[b]pale to red muscles around 1-3 cm in the superficial muscles at the injection site and the small droplets of vaccine left.

[c]red and inflammable muscles around 3-4 cm in the superficial and deep muscles at the injection sites.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Part 1: Effects of feed additive containing polysaccharide gel (PG) from fruit-rind of *Durio zibethinus* Murr. on immune response and cholesterol levels in plasma and muscle of chickens.

The results was compared between chickens fed commercial feed with PG in different ratios and chickens fed with commercial feed without PG in aspects of body weight gains, antibody titers, H:L ratio, total bacteria count and *Salmonella* in chicken feces and cholesterol in plasma and muscle.

1. Impact of PG on feed conversion ratio.

The body weight gains of chickens in the control group were not significantly lower than those of the treatment groups. Therefore, the PG in feed did not provide an adverse effect to the chickens. The results were in accordance with Pholdaeng et al. (2005), the PG from Durian fruit-rind added to the shrimp diet did not affect the growth of shrimp.

2. The effect of PG on hemagglutination-inhibition (HI) titers against Newcastle disease (ND) and ELISA titers against infectious bursal disease (IBD).

At 6-week-old, the chickens in the group received 3 g% PG revealed the highest antibody titers against ND and IBD immunity which were significantly higher than the other groups ($P<0.05$) (Figure 4-1 and 4-2). This was in accordance with Pholdaeng et al. (2005) who revealed that PG can stimulate the immune responses in

shrimp. Also Chansiripornchai et al. (2008) reported that chickens fed with PG showed a potentially better immunity against ND than non PG feeding group.

3. The effect of PG on the ratio of Heterophil:Lymphocyte (H:L ratio).

No significant difference was found in the result of H:L ratio between the experimental groups fed with PG and the control group (Figure 4-3). The H:L ratio is the ratio of heterophil on lymphocytes. An alteration of this value depends on the types, ages of animals and environment changes. When the chickens were stressed, glucocorticoid hormone was increased resulting in a decrease of lymphocytes (Puvadolpirod and Thaxton, 2000). In contrast to lymphocytes, when the heterophil increased, the H:L ratio also increased. Normal range of the H:L ratio of poultry is 0.33-0.5. In this experiment, the H:L ratios were rather high at ranges 0.36-11.85 (Figure 4-1) which might have been caused by the disturbance of the chickens from frequently serum collection and weighing in every week. Weighing chickens every week can cause stress, so the glucocorticoid hormone will increase and also the lymphocytes will decrease. At 5-week-old, the H:L ratios of the chickens in all groups were highly increased. It might be caused by the noise from building work, however, the chickens fed with 0 % PG showed the highest a higher H:L ratio than those chickens in the experimental groups. Thus, the chickens fed with a PG diet tended to have a better ability to resist stress than those chickens fed with a non PG diet at week 5.

4. The effect of PG on the total bacteria count and the colonies number of *Salmonella* in chicken feces.

At 5 and 6-week-old, the total bacteria and *Salmonella* counts in chicken feces of the control group were significantly higher than the experimental groups ($P<0.05$) (Table 4-3 and 4-4). The PG reduced *Salmonella* in chicken feces even at the lowest amounts of PG (1 g% PG). The PG has an antibacterial activity against both gram positive and gram negative bacteria (Lipipan et al., 2002; Pholdaeng et al., 2005;

Pholdaeng and Pongsamart, 2010). Therefore, the PG could reduce bacteria in chicken feces and the higher PG in the diet showed the better reduction of the total bacteria count in the feces. The PG was not absorbed through the stomach (Tippayakulet al., 2002), so it passed through the small intestine where it was firstly infected with *Salmonella* (Bangtrakulnonth, 2002). In addition, the physical properties of PG include intrinsic acid condition with pH at 2.2-2.6 due to its acidic sugar component, especially galacturonic acid. The mechanism of inhibition of bacteria by PG may be related to the property of its acidic polygalacturonic acid chain in addition to adhesion reaction with neutral sugar side chains in the pectic polysaccharide (Hokputsa et al., 2004). The PG has intrinsic viscosity and adhesive properties due to electronegativity and branch chain neutral sugars of the pectic polysaccharide, which probably adhesively bind on the cell's outer surface (Lipipan et al., 2002; Pongsamart et al., 2005). Also, the PG may cause adhesion interference to the cell's normal function and alters the membrane permeability (Tsai et al., 1999). For this reason, the PG could inhibit *Salmonella* and reduce the amount of total bacteria in chicken.

5. The effect of PG on the plasma and muscle cholesterol in chickens.

No significant difference in cholesterol in the chickens' plasma between the control and the experimental groups fed commercial feed with PG were observed (Figure 4-4). However, the chickens fed commercial feed with PG tended to have a lower cholesterol level in the plasma than the chickens fed with diet without PG feed. This result agreed with Chansiripornchai et al. (2008) previously reported that mixing the PG in the chicken diet could reduce the cholesterol level in serum.

The cholesterol levels in the muscle of chickens fed with 3 g% PG was significantly lower than those of chickens in the control group ($P<0.05$) (Table 4-5). According to Chansiripornchai et al. (2008), the PG in feed can reduce the level of cholesterol in muscle measured by the colorimetric method. Although the analysis method was different in this case, the result was the same. It was confirmed that the PG

in the chicken diet potentially reduced the cholesterol in the chickens' muscle. The PG can well confine liquid such as cholesterol and fatty acid by absorbing lipids and cholesterol within the PG molecule during pass through the alimentary canal resulting in the low absorption of lipids and cholesterol in chickens (Tippayakul et al., 2002). Moreover, the nutrient absorption rate depends on the rate at which nutrients are in contact with the absorptive epithelium layer (Pholdaeng and Pongsamart, 2010). Thus, such a high PG concentration may cause interference in the rate of nutrient absorption. Increased viscosity of the diet can likely retard nutrient absorption in the same way as the effect of dietary fiber is not absorbed in the stomach (Fair et al., 1980). Too high level of PG in the diets may not be appropriate because of the retardation of nutrient absorption in the digestive tract. For this reason, the cholesterol in chickens fed a diet with PG at the high concentration was lower than that of chicken fed a diet without PG. Therefore, PG can reduce cholesterol in muscle of chickens.

The PG may be used as a feed additive to broiler chickens in order to reduce contaminated bacteria in gastrointestinal tract and also decrease the cholesterol in chickens' muscle. The low cholesterol of meats have benefit for patients with hypercholesterol or required low fat diets. Moreover, bactericidal activity of PG in chicken feed can probably reduce the highly uses of antibiotics in poultry.

Part 2: Effects of polysaccharide gel (PG) from fruit-rind of *Durio zibethinus* Murr. as an vaccine adjuvant.

The experiment was compared the chickens between the negative control group, the positive control group and the group that received inactivated vaccine contained 0.5, 1.0 and 1.5 % PG as an adjuvant and the group that received commercial inactivated ND vaccine in aspects of FCR and antibody titers.

1. **The effect of NDV vaccine on FCR**

The FCR of chickens in the control group were not significantly different when compared with the treatment groups and the commercial group. From the result show that the inactivated vaccine with 0.5, 1.0 and 1.5 % PG was had been potentially in preventing disease as well as the commercial vaccine.

2. **The effect of PG in chickens receiving different concentrations of PG containing in inactivate ND vaccines on hemagglutination-inhibition (HI) titers against Newcastle disease (ND).**

After vaccine injection at week 3, the immune response was evaluated by testing HI antibody titer against NDV at week 4, 5 and 6, respectively. At week 4 HI titer in control group and treated group injected with inactivated vaccine with 0.5, 1.0 and 1.5 % PG as well as commercial inactivated ND vaccine were not significant. At week 5 and 6 the HI titer in negative control, positive control and treated group injected with 0.5 and 1 % PG were not significant difference, but these values were lower than those group injected with 1.5 % PG and commercial inactivated ND vaccine and these 2 groups showed not significant difference in their HI titer. In conclusion, the HI antibody titer of chickens receiving commercial inactivated ND vaccine was highest than the other group. These results can be explained by the fact that the mixing process of vaccine preparation for making emulsion may take effect on this aspect because of a better quality of emulsifying process of the commercial vaccine. The formulas for the vaccine companies are not disclosed. Vaccine formulation is important to come up with the antibody level. Fukanoki et al. (2000) reported the dilution of the aqueous phase in the formula can reduce the rate of release of vaccine antigens and antibodies to a higher level. Therefore, the formula for the preparation of vaccines, it is important to come up with the antibody. If the vaccine has been modified to provide and improve mixing, it will help to prepare the vaccine has comparable performance with the commercial vaccine.

3. The viscosity, stability and tissue reaction tests

The viscosity test revealed that the commercial inactivated ND vaccine provided high viscosity than the inactivated vaccine without PG, 1.0 % PG, 0.5 % PG and 1.5 % PG, respectively. However, in practice it did not see the difference. When measured at the chickens vaccinated in each group were similar. The advantage of less viscosity vaccine is benefit to practical vaccination and the cleaning of vaccine equipment.

All vaccine preparations showed very good emulsion after preparation. At 4 weeks of observation at a 4°C and 25°C the inactivated vaccine with 1.5 % PG showed less stability than the other groups. Separation had been found between oil and aqueous phase but it returned to homogeneous again after shaking.

The study found that the use of vaccines prepared from inactivated vaccine with 0.5 and 1.0 % PG can be effective in the prevention of disease as well as the use of commercial vaccines.

The PG may be used as a adjuvant in inactivated ND vaccine to broiler chickens for immunostimulation.

REFERENCES

Abraham, Z. D.; and Mehta, T. Three-week psyllium-husk supplementation:effect on plasma cholesterol concentrations, fecal steroid excretion, and carbohydrate, absorption in men. Am. J. Clin. Nutr. 47 (1988): 67-74.

Alexander, D. J. Newcastle disease diagnosis. In: Newcastle disease. London: Kluwer Academic Publishers. 1988: 147-160.

Alexander, D. J. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Off. Int. Epiz. 19 (2000): 443-462.

Alexander, D. J.; Calnek, B. W.; Barnes, H. J.; Beard, C. W.; McDougal, L. R.; and Saif, Y. M. Newcastle Disease and Other Paramyxovirus infections. In: Diseases of Poultry. 10th ed., (eds.), Ames, IA:Iowa State University Press. 1997: 541-569.

Allan, W. H.; and Gough, R. E. A standard haemagglutination inhibition test for Newcastle disease Vaccination and challenge. Vet. Rec. 95 (1974): 147-149.

Anderson, J. W.; Baird, P.; and Davis, R. H. Health benefits of dietary fiber. Nutr. Rev. 67 (2009) (4): 188–205.

Araki, N. S.; Horiuchi, A.; Rahim, T.; and Morino, Y. Microquantification of cholesterol and cholesteryl esters in rat peritoneal macrophages by reverse-phase high-performance liquid chromatography. Anal. Biochem. 185 (1990): 339-345.

Ascherio, A.; and Willett, W. C. Health effects of trans fatty acids. Am. J. Clin. Nutr. 66 (1997): 1006S–1010S.

Bangtrakulnonth, A. Protocols for isolation, identification, serotyping and susceptibility testing of *Salmonella*. A global *Salmonella* surveillance and laboratory support project of the world Health Organization, Department of Communicable Disease surveillance and Response (CSR). In collaboration with Centers for Disease control and prevention, USA, Danish Veterinary Laboratory, Denmark and national Salmonella and Shigella Center, Thailand. 2002: 39-56.

Behrman, E. J.; and Gopalan, V. Cholesterol and Plants. J. Chem. Educ. 82 (12) (2005): 1791.

Bennejean, G.; Guittet, M.; PicaultI, J. P.; Bouquet, J. F.; Devaux, B.; Gaudry, D.; and Moreau, Y. Vccination of one-day-old chicks against newcastle disease using inactivated oil adjuvant vaccine and/or live vaccine. Avian. Pathol. 7 (1978): 15-27.

Boerjan, W.; John, R.; and Marie, B. Ligninbiosynthesis. Annu. Rev. Plant Biol. 54 (2003): 519 –46.

Brown, M. J. Durio — A Bibliographic Review. International Plant Genetic Resources Institute (IPGRI). 1997: 35.

Brody, T. Nutrients that resist or escape digestion. Nutritional Biochemistry., 2nd ed. USA: Academic Press, 1999.

Brunzell, J. D.; Davidson, M.; Furberg, C. D.; Goldberg, R. B.; Howard, B. V.; Stein, J. H.; an Witztum, J. L. Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care. 31(4) (2008): 811–22.

Burkill, I. H.; and Haniff, M. Malay village medicine, prescriptions collected. Gardens Bulletin Straits Settlements. (6) (1930): 176–177.

Center for agricultural information office of agricultural economics ministry of agriculture and co-operatives Bangkok, Thailand. Agricultural statistic of Thailand. 410 (2004): 48.

Chansiripornchai, N.; and Sasipreeyajan, J. Efficacy of live B1 or Ulster 2C Newcastle disease vaccines simultaneously vaccinated with inactivated oil adjuvant vaccine for protection of Newcastle disease virus in broiler chickens. Acta Vet. Scand. 48 (2006): 1-4.

Chansiripornchai, N.; Chansiripornchai, P.; and Pongsamart, S. A preliminary study of polysaccharide gel extracted from the fruit-hulls of Durian (*Durio zibethinus*

Murr.) on immune responses and cholesterol reduction in chicken. Acta Hort. 786 (2008): 57-60.

Chansiripornchai, N.; and Sasipreeyajan, J. Comparison of the efficacy of the immune complex and conventionally live vaccine in broilers against infectious bursal disease infection. Thai J. Vet. Med. 39 (2) (2009): 115-120.

Chansiripornchai, P.; Pongsamart, S.; Nakchat, O.; Pramatwinai, C.; and Rangsipipat, A. The efficiency of polysaccharide gel extracted from fruit-hulls of durian (Durio zibethinus L.) for wound healing in pig skin. Acta Hortic. 679 (2005): 37-43.

Christie, W. Lipid analysis: isolation, separation, identification, and structural analysis of lipids. Ayr, Scotland: Oily Press. 2003.

Cleave, T. L. The neglect of natural principles in current medical practice. J. Royal Navy Medical Services. 42 (1956): 55–83.

Corrier, D. E.; Hollister, A. G.; Nisbet, D. J.; Scanlan, C. M.; Beierand, R. C.; and DeLoach, J. R. Competitive exclusive of Salmonella Enteritidis in Leghorn chicks:comparison of treatment by crop gavage, drinking water, spray, or lyophilized alginate beads. Avian Dis. 38 (2) (1994): 297-303.

Donald, V.; and Judith, G. V. Sugars and Polysaccharides. Biochemistry. 2nd ed. USA. John Wiley & Sons Inc. 1995.

Dumitriu, S. Polysaccharide in medical application. New York, USA. Marcel Dekker. 1996.

Durrington, P. Dyslipidaemia. Lancet. 362 (2003): 717–31.

Eastwood, M. A.; Brydon, W. G.; and Tadesse, K. Effect of fiber on colon function. In Kay R, Spiller GA Medical aspects of dietary fiber. New York: Plenum Medical Book Co. 1980: 1–26.

Eterradossin, N.; Rivallan, G.; Toquin, D.; and Guitte, M. Limited antigenic variation among recent infectious bursal disease virus isolates from France. Arch. Virol. 142 (1997): 2079–2087.

Gast, R. K. Paratyphoid infections. In: B.W. Calnek (ed.). Diseases of poultry. 10th ed. USA. 1997: 97-121.

Greger, J. L. Nondigestible carbohydrates and mineral bioavailability. J. Nutr. 1999: 129.

Girddit, W.; Tippayakul, C.; Lertchaiporn, J.; Sutanthavibul, N.; and Pongsamart, S. Characterization and properties of polysaccharide fiber from durian fruit-hull. Proceedings of 27th Congress on Science and Technology of Thailand, 16-18 October, 2001, p. 576. Lee Garden Plaza Hotel, Hat Yai, Songkla, Thailand, 2001.

Gordon, D. J.; Probstfield, J. L.; Garrison, R. J.; Neaton, J. D.; Castelli, W. P.;Knoke, J. D.; Jacobs, D. R.; Bangdiwala, S.; and Tyroler, H. A. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 7 (1) (1989): 8–15.

Fair, P. H.; Fortner, A. R.; Millikin, M. R.; and Sick, L. V. Effects of dietary fiber on growth, assimilation and cellulose activity of the prawn (Macrobrachium rosenbergii) In: Proceeding 11th Annual Workshop World Mariculture Society, pp. 359-381. 1980.

Faylon, P. S. Durian: Distinct Taste, Preferred Sweet. Philippine Council for Agriculture, Forestry and Natural Resources Research and Development. 2005.

Folch, J.; Less, M.; and Stoanestamley, G. H. A Simple method for isolation and purification of total lipid from animal tissues. J. Biol. Chem. 226 (1957): 497-509.

Freudenheim, J. L. Risks associated with source of fiber and fiber components in cancer of the colon and rectum. Cancer Res. 50 (1990): 3295-3300.

Fukanoki, S.; Iwakura, T.; Iwaki, S.; Matsumoto, K.; Takeda, R.; Ikeda, K.; Shi, Z.; and Mori, H. Safety and efficacy of water-in-oil-in-water emulsion vaccines containing Newcastle disease virus hemagglutination-neuraminidase glycoprotein. Avian Path. 30 (5) (2001): 509-516.

Haines, T. H. Do sterols reduce proton and sodium leaks through lipid bilayers. Prog. Lipid Res. 40 (4) (2001): 299–324.

Hanukoglu, I. Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. J. Steroid. Biochem. Mol. Biol. 43 (8) (1992): 779–804.

Hipsley, E. H. Dietary fibre and pregnancy Toxaemia. Br. Med. J. 1953: 420.

Hokputsa, S.; Gerddit, W.; Pongsamart, S.; Inngjerdingen, K.; Heinze, T.; Koschella, A.; Harding, S.; and Paulsen, B. Water-soluble polysaccharides with pharmaceutical importance from durian rinds (*Durio zibethinus* Murr.): isolation, fractionation, characterisation and bioactivity. Carbohyd. Polym. 56 (2004): 471-481.

Hong, S.; Lin, H.; Ralph, L. P.; and Fernandez, M. L. Dietary soluble fiber lowers plasma LDL cholesterol altering lipoprotein metabolism in female guinea pigs. J. Nutr. 128 (1998): 1434-1441.

Hoving, E. B. Chromatographic methods in the analysis of cholesterol and related lipids. J. Chromatogr. 671 (1995): 352-362.

Incardona, J. P.; Eaton, S. Cholesterol in signal transduction. Curr. Opin. Cell Biol. 12(2) (2000): 193–203.

ISO, 2002. International Organization of Standardization 6579:2002. Horizontal method for the detection of *Salmonella* spp., Geneve.

Jensen, R. G.; Hagerty, M. M.; and McMahon, K. E. Lipids of human milk and infant formulas: a review. Am. J. Clin. Nutr. 31(6) (1978): 990–1016.

JIRR, B. High-performance liquid chromatographic determination of cholesteryl esters in the blood of obese children. J. Chromatogr. 571 (1991): 19-28.

Kajiwara, K.; Miyamoto, T. Progress in structural characterization of functional polysaccharides. In S. Dumitriu, and M. Dekker (eds.), Polysaccharide: structural diversity and functional versatility, The United States of America. 1998.

Krebs, H.; Decker, K.; and Feodor, L. 6 April 1911-6 August 1979. Biographical Memoirs of Fellows of the Royal Society. 28(1982): 260.

Lewington, S.; Whitlock, G.; Clarke, R.; Sherliker, P.; Emberson, J.; Halsey, J.; Qizilbash, N.; Peto, R.; and Collins, R. Blood cholesterol and vascular mortality by age,

sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 370 (2007): 1829–39.

Lewis, G. F.; and Rader, D. J. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ. Res. 96 (12) (2005): 1221–32.

Lipipan, V.; Nantawanit, N.; and Pongsamart, S. Antimicrobial activity (in vitro) of polysaccharide gel from durian fruit-hulls. Songklanakarin. J. Sci. Technol. 24 (2002): 31-38.

Lukert, P. D.; and Saif, Y. M. Infectious bursal disease. In: Y.M. Saif (Ed.) Disease of Poultry. 11th Ed., Iowa State University, Ames, Iowa, USA. 2003: 161-179.

Mensink, R. P.; and Katan, M. B. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arteriosclerosis and thrombosis. AHA Journal. 12 (8) (1992): 911–919.

Nakchat, O.; Birksawan, P.; Komin, K.; Chuthatep, S.; Pramatwinai, C.; and Pongsamart, S. Effect of durian gel dressing on wound healing in pig skin. Proceeding of the 28th Congress on Science and Technology of Thailand. October 2002, p. 466. Qeen Sirikit Nation Convention Center, Bankok, Thailand. 2002.

Norie, A. Microquantification of cholesterol and cholesteryl esters in rat peritoneal macrophages by reverse-phase high-performance liquid chromatography. Anal. Biochem. 185 (1990): 339-345.

Nugent, A. P. Health properties of resistant starch. Nutrition Bulletin. 30(1) (2005): 27-54.

O'Gara, E.; Guest, D. I.; and Hassan, N. M. Botany and Production of Durian (Durio zibethinus) in Southeast Asia. In Drenth, A. and Guest, D. I. Diversity and management of Phytophthora in Southeast Asia. ACIAR Monograph Australian Centre for International Agricultural Research (ACIAR) 114 (2004): 180–186.

Ostlund, R. E.; Racette, S. B.; and Stenson, W. F. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am. J. Clin. Nutr. 77(6) (2003): 1385–1589.

Pawlina, W.; and Ross, M. W. Histology: a text and atlas: with correlated cell and molecular biology. Philadelphia: Lippincott Wiliams & Wilkins. 230(2006)

Pholdaeng, K.; Withyachumnarnkul, B.; and Pongsamart, S. Effect of antibacterial durian polysaccharide additive diet on immune response in the black tiger shrimp (*Penaeus monodon*). Proceeding of the 31st Congress on Science and Technology of Thailand, October 18-20, p.264. Nakhon Ratchasima. 2005.

Pholdaeng, K.; and Pongsamart, S. Studies on the immunomodulatory effect of polysaccharide gel extracted from Durio zibethinus in *Penaeus monodon* shrimp against *Vibrio harveyi* and *WSSV*. Fish Shellfish Immun. 28(2010): 555-561.

Pongsamart, S.; and Panmaung, T. Isolation of polysaccharide from fruit-hulls of durian (*Durio ziberthinjus* L.). Songklanakarin J. Sci. Technol. 20(3) (1998): 323-332.

Pongsamart, S.; Sukrong, S.; and Tawatsin, A. The determination of toxic effects at a high oral dose of polysaccharide gel extract from fruit-hulls durian (*Durio zibethenus* Murr.) in mice and rats. Songklanakarin J. Sci. Technol. 23(1) (2001): 55-62.

Pongsamart, S.; Tawatsin, A.; and Sukrong, S. Long-term consumption of polysaccharide gel from durian fruit-hulls in mice. Songklanakarin J. Sci. Technol. 24 (2002): 555-567.

Pongsamart, S.; Lipipan, V.; Nantawanit, N.; and Lertchaiporn, J. Novel water soluble antibacterial dressing of durian polysaccharide gel. Acta Hort. 678 (2005): 65-73.

Prosky, L.; and De Vries, J. W. Properties of food fibers/fibers in food product. Controlling Delivery Fiber in Food Product. New York: Van Nosrand Reinhold. 1992: 6-10.

Puvadolptrod, S.; and Thaxton, J. P. Model of physiological stress in chickens. 1. Response parameter. Poult. Sci. 78 (2000): 370-376.

Rhodes, C.; Lubert, S.; and Tasker, R. Biochemistry (4th ed.). San Francisco: W.H. Freeman. 280 (1995): 703.

Sadava, D.; Hillis, D. M.; Heller, H. C.; and Berenbaum, M. R. Life: The Science of Biology. 9th Edition. San Francisco: Freeman. 2011: 105–114.

Scott, B. R.; and Leo, S. J. Overestimation of the cholesterol content of eggs. J. Agric. Food. Chem. 37 (1989): 917-920.

Seta, K., Nakamura, H.; and Okuyama, T. Determination of ∝-tocopherol, free cholesterol, esterified cholesterols and triacylglycerols in human lipoproteins by high-performance liquid chromatography. J. Chromatogr. 515 (1990): 585-595.

Sithipirojsakul, S.; Sutantavibul, N.; and Pongsamart, S. Application of powdered cellulose from durian fruit-hulls for preparation of direct compression tablets. Thai J. Pharm. Sci. 26 (3-4) (2002): 160.

Siralertmukul, K.; Khunton, S.; Suwanno, N.; and Pongsamart, S. Production of carboxymethyl cellulose from durian hull. Proceeding of the 31th Congress on Science and Technology of Thailand, October 18-20, 2005, p.189. Nakhon Rachasima, Thailand. 2005.

Smith, L. L. Another cholesterol hypothesis: cholesterol as antioxidant. Free Radic. Biol. Med. 11(1) (1991): 47–61.

Smith, F.; and Montgonery, R. Chapter 5 : Separation of mixtures of polysaccharides. The Chemistry of Plant Gums and Mucilages. London. Chapman & Hall Ltd. 1959: 85.

Spradbrow, P. B. Newcastle disease — an overview. In: Copland, J.W. ed. Newcastle disease in poultry: a new food pellet vaccine. Canberra, ACIAR monograph. 5 (1987): 12–18.

Stone, H. D. Newcastle disease oil emulsion vaccines prepared with animal, vegetable and synthetic oils. Avian Dis. 41(3) (1997): 591-597.

Subhadrabandhu, S.; and Ketsa, S. Durian: King of tropical fruit. Throdon, Wellington, New Zealand: Daphne Brasell Associates. 2001: 1-9.

Terpstra, A. H. M.; Lapre, J. . A.; DeVries, H. T.; and Beynen, A. C. Dietary pectin with high viscosity lowers plasma and liver cholesterol concentration and plasma

cholesteryl ester transfer protein activity of hamsters. J. Nutr. 128 (1998): 1944-1949.

Tippayakul, C.; Piyasirananda, W.; and Pongsamart, S. Evaluation (*in vitro*) of durian gel in trapping of lipid and sugar to assess its potential use as dietary control. Proceedings on 28th Congress on Science and Technology of Thailand. October 24-26, 2002, p. 331. Queen Sirikit Nation Convention Center, Bankok, Thailand. 2002

Trowell, H. Dietary fibre and coronary heart disease. Revue Européenne d' Etudes Cliniques et Biologiques. 17(4) (1972): 345-349.

Tsai, G. J.; and Su, W. H. Antibacterial activity of shrimp chitosan against Escherichia coli. J. Food Protect. 62 (1999): 239-243.

Tymoczko, J. L.; Stryer, L.; and Berg, J. M. Biochemistry. San Francisco: W.H. Freeman. 2002: 726–727.

Tungland, B. C.; and Meyer, D. Nondigestible oligo- and polysaccharides (dietary fiber): their physiology and role in human health and food. Comp. Rev. Food. Sci. Food. Safety. 3(2002): 73-92,

Umpray, K.; Chanpaparp, K.; and Pongsamart, S. The studies of Durian Rind Extracts as an Aqueous Binder I:Evaluation of Granule Proties. Th. J. Pharm. Sci. 15(2) (1990): 95-115.

Umpray, K.; Kaitmonkong, R.; and Pongsamart, S. Evaluation of Tablet Disintegrating Properties of Durian Rind Extracts. Proceedings of NUS-JSPS Seminar, October 23-26, Chiba, Japan. 1990.

van den Berg, T. P. Acute infectious bursal disease in poultry. A review. Avian Path. 29 (2000): 175-190.

Westheimer, F. H.; Lipscomb, W.; and Konrad, B. Biographical Memoirs of Fellows of the Royal Society, 21 January 1912 - 5 October 2000, p. 43. 2002.

Weingärtner, O.; Pinsdorf, T.; Rogacev, K. S.; Blömer, L.; Grenner, Y.; Gräber, S.; Irich, C.; Girndt, M.; Böhm, M.; Fliser, D.; Laufs, U.; Lütjohann, D.; and Heine, G. H.

Federici, Massimo. ed. The relationships of markers of cholesterol homeostasis with carotid intima-media thickness. Plos. One. 5 (10) (2010): 13467.

Whiteman, C. E.; and Bickford, A. A. Newcastle disease. In Avian Disease Manual 2nd edition. Edited by: Whiteman CE, Bickford AA. The American Association of Avian Pathologist, Pensylvania, Kennett square. 1983: 55-56.

Xiong, H.; Slavik, M. F.; and Walker, J. T. Spraying chicken skin with selected chemicals to reduce attached *Salmonella Typhimurium*. J. Food. Protect. 61 (1998): 272-275.

Yeagle, P. L. Modulation of membrane function by cholesterol. Biochimie. 73(10) (1991): 1303–1310.

Zlatkis, A.; Zak, B.; Boyle, A. J.; and Mich, D. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41 (1953): 486 – 492.

Appendix

Alsever's solution Volume 1 liter

Dextrose	20.5	g
Sodium citrate	8	g
Citric acid	0.55	g
NaCl	4.2	g
Distilled water add to	1,000	ml

Dissolve all chemical substances in distilled water and sterilize by autoclaving at 121° C for 10 minutes.

PBS (Phosphate Buffered Saline) pH 7.0 Volume 1 liter

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.25	g
Distilled water add to	1,000	ml

Sterilize by autoclaving at 121° C for 15 minutes.

NSS (Normal Saline Solution) Volume 1 liter

Nacl	9	g
Distilled water add to	1,000	ml

Sterilize by autoclaving at 121° C for 15 minutes.

BPW (Buffered Peptone Water) pH 7.5 Volume 1 liter

Peptone	10	g (Gelysate™, USA.)
NaCl	5	g
Na2HPO4.12H2O	9	g
KH_2PO_4	1.5	g
Distilled water add to	1,000	ml

Sterilize by autoclaving at 121° C for 15 minutes.

XLT4 (Xylose-Lysine-Tergitol 4) (Merck KgaA, Darmstsdt, Germany) Volume 1 liter

XLT4 base	59	g
XLT4 supplement	4.6	mg
Distilled water add to	1,000	ml

Add XLT4 supplement at 50°C, do not autoclave.

BIOGRAPHY

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