

Effect of Polysaccharide Gel Extracted from *Durio zibethinus* Rind on Immune Responses, Bacteria Counts and Cholesterol Quantities in Chickens

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Abstract

Polysaccharide gel (PG) from the rind of durian (*Durio zibethinus* Murr.) is a soluble powder that gives good results as an excipient in pharmacological and food preparation. The aim of the present study was to evaluate the effects of PG as a feed-supplement diet on body weight gain, immune stimulation, total bacteria and Salmonella in feces, and cholesterol levels in broilers. Eighty, one-day-old broiler chicks were divided into 4 groups. Three experimental groups were fed a commercial diet coated with PG 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 in the treatment and control groups was not significantly different. At six weeks old, the hemagglutination inhibition and ELISA antibody titers against Newcastle disease (ND) virus and infectious bursal disease (IBD) virus, respectively, were significantly different ($p < 0.05$). The chickens fed commercial feed with 3 g/100 g PG revealed the highest antibody titers against the ND and IBD. There was no significant difference in heterophil : lymphocyte ratio between the treatment groups and the control group. The total bacteria count in chicken feces was significantly reduced, 81-97%, in the experimental groups compared to the control group ($p < 0.05$). Moreover, Salmonella suspected colonies were significantly reduced in the experimental groups compared to the control group ($p < 0.05$) and no Salmonella suspected colonies were detected in the experimental groups. The cholesterol levels in the plasma of the chickens in the treatment groups were lower than those of the control group. Furthermore, the cholesterol content of the muscles of the broilers fed on the diet with 3 g/100 g PG was significantly lower than those of the chickens fed on the diet without PG ($p < 0.05$). Therefore, polysaccharide gel in the diet benefited health promotion in broiler chickens as an antibacterial activity, immunostimulant and in cholesterol reduction.

Keywords: cholesterol, dietary fiber, immunostimulant, polysaccharide gel

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บทคัดย่อ

ผลของสารสกัดเจลพอลิแซ็กคาไรด์จากเปลือกทุเรียน *Durio zibethinus* ต่อการตอบสนองทางภูมิคุ้มกัน จำนวนแบคทีเรียและปริมาณโคเลสเตอรอลในไก่

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พอลิแซ็กคาไรด์เจล (PG) จากเปลือกของผลทุเรียน (*Durio zibethinus* Murr.) มีลักษณะเป็นผงละลายน้ำได้ซึ่งเป็นกระสายยาที่ดีในทางเภสัชวิทยาและการเตรียมอาหาร การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของ PG ในฐานะอาหารเสริม ในด้านการเพิ่มน้ำหนักตัว การกระตุ้นภูมิคุ้มกัน จำนวนแบคทีเรียโดยรวมและซัลโมเนลลาในมูลของไก่เนื้อ และการลดโคเลสเตอรอลในไก่เนื้อ ไก่เนื้ออายุ 1 วัน จำนวน 80 ตัว แบ่งออกเป็น 4 กลุ่ม คือ 3 กลุ่มทดลองที่ได้รับอาหารที่จำหน่ายเชิงพาณิชย์ที่เคลือบด้วย PG ขนาด 1, 2 และ 3 กรัม/100 กรัม ตามลำดับ และกลุ่มควบคุมที่ได้รับอาหารที่จำหน่ายเชิงพาณิชย์ที่ไม่เคลือบ PG จากการศึกษาเป็นเวลา 42 วัน ไม่พบความแตกต่างอย่างมีนัยสำคัญของน้ำหนักไก่ที่เพิ่มขึ้นระหว่างกลุ่มทดลองและกลุ่มควบคุม เมื่อไก่อายุ 6 สัปดาห์ พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ของแอนติบอดีไตเตอร์ต่อวิธีการยับยั้งการตกตะกอนเม็ดเลือดแดงและวิธีฮีโมไลซิสต่อไวรัสนิวคาสเซิล (ND) และไวรัสเบอร์ซาอิกเสบติดต่อ (IBD) ตามลำดับ โดยไก่ที่ได้รับอาหารเชิงพาณิชย์ที่เคลือบ PG ขนาด 3 กรัม/100 กรัม มีระดับแอนติบอดีไตเตอร์สูงสุดต่อ ND และ IBD ไม่พบความแตกต่างอย่างมีนัยสำคัญของ heterophil : lymphocyte ratio ระหว่างกลุ่มทดลองที่ได้รับอาหารที่มี PG และกลุ่มควบคุมที่ได้รับอาหารที่ไม่มี PG พบว่าจำนวนแบคทีเรียในมูลไก่ลดลงอย่างมีนัยสำคัญทางสถิติ คือ ระหว่างร้อยละ 81-97 เมื่อเปรียบเทียบกับระหว่างไก่กลุ่มทดลองและกลุ่มควบคุม ($p < 0.05$) นอกจากนี้ พบว่าจำนวนโคโลนีที่สงสัยว่าเป็นซัลโมเนลลาลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับระหว่างไก่กลุ่มทดลองและกลุ่มควบคุม ($p < 0.05$) และไม่พบโคโลนีที่สงสัยว่าเป็นซัลโมเนลลาในกลุ่มทดลอง ระดับโคเลสเตอรอลในพลาสมาในไก่กลุ่มทดลองมีระดับต่ำกว่าไก่กลุ่มควบคุม นอกจากนี้ปริมาณโคเลสเตอรอลในกล้ามเนื้อของไก่ที่ได้รับ PG 3 กรัม/100 กรัม มีระดับต่ำกว่าไก่กลุ่มควบคุมที่ไม่ได้รับ PG อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ดังนั้น PG ในอาหารมีประโยชน์ในการส่งเสริมสุขภาพไก่เนื้อในด้านการต้านแบคทีเรีย การกระตุ้นภูมิคุ้มกันและการลดลงของโคเลสเตอรอล

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Introduction

Polysaccharides extracted from the rind of durian (*Durio zibethinus* Murr.), the most popular fruit of Thailand, were studied. Polysaccharides are composed of soluble fiber (polysaccharide gel) and insoluble fiber (polysaccharide fiber) (Girddit et al., 2002; Hokputsa et al., 2004). Polysaccharide gel (PG) is composed of long chain α (1 \rightarrow 4) linked polygalacturonan with side chains of neutral sugars with terminal non-reducing end fructose and glucan (Hokputsa et al., 2004) whereas insoluble polysaccharide fiber is composed of long chain glucan as α -cellulose (Umpray et al., 1990). Polysaccharide gel can be absorbed and slowly dissolves in water. The gelling properties of PG are of benefit for utilizing as pharmaceutical aids. The soluble and insoluble

durian polysaccharides are useful for tableting (Sithipirojsakul et al., 2002). Polysaccharide gel is resistant to acid and enzyme α -amylase hydrolysis. Polysaccharides cannot be completely digested and absorbed in the gastrointestinal tract. No toxic effects have been found in acute and subchronic toxicity studies, which confirm the consumptive safety of durian polysaccharides (Pongsamart et al., 2002). The immunomodulating properties of PG can be estimated by complement fixation assay (Hokputsa et al., 2004). Polysaccharide gel has a beneficial effect in improving the immune systems 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, stimulated the humoral immune responses and could reduce cholesterol in the pectoral muscles of chickens. According to

Chansiripornchai et al. (2008), PG in feed can reduce the level of cholesterol in muscle measured by the colorimetric method. Polysaccharide gel can well confine liquid such as cholesterol and fatty acid by absorbing lipids and cholesterol within PG molecule when passing through the alimentary canal, resulting in the low absorption of lipids and cholesterol in chickens (Tippayakul et al., 2002). Moreover, nutrient absorption rate depends on the rate at which nutrients are in contact with the absorptive epithelium layer (Beseres et al., 2005; Pholdaeng and Pongsamart, 2010). Thus, such a high PG concentration may cause interference in the rate of nutrient absorption. Increased viscosity of a diet can likely retard nutrient absorption in the same way as the effect of dietary fiber that is not absorbed in the stomach (Fair et al., 1980). Currently, broilers are not only produced at an industrial level but also at small holding level. Small holders serve as an important source of food supply in remote and poor areas. An outbreak of infectious disease (such as Newcastle disease, Infectious bursal disease, etc.) certainly causes severe economic damage to all levels of broiler production. Many pathogenic bacteria are found in chicken feces, such as coliform, *Salmonella* and pathogenic *E. coli*. Generally, bacteria in feces are 100-1000 times of anaerobic bacteria compared to aerobic bacteria and most pathogenic bacteria are aerobic bacteria (Simon and Gorbach, 1984). The procedure of total bacteria count only focuses on the aerobic bacteria and this method generally characterized the contaminated or pathogenic bacteria in feces. The present study aimed to investigate the effect of PG as a feed additive on body weight gain, immunostimulation, reduction in meat and plasma cholesterol and also reduction in fecal bacteria in chickens fed commercial feed with PG in different ratios and chickens fed commercial feed without PG.

Materials and Methods

Polysaccharide gel extraction from rind of durian: Extraction of PG was previously reported (Pongsamart and Panmaung, 1998). Briefly, polysaccharide gel was extracted from the dried rind of durian with boiling water. The polysaccharide gel extracted water was concentrated under reduced pressure and precipitated by its addition into acidified aqueous ethanol, filtered, dried and ground. The pale beige colored powder of durian polysaccharide was used in the experiment.

Preparation of polysaccharide gel feed-supplement diet: To make a ten percent PG stock feed additive diet, 10 g of the purified PG was dispersed in water and then spray-coated onto 100 g of feed pellets of a commercial broiler diet (Betagro, Thailand), in a pan coating machine (Yinrich, China). To make one, two and three g% PG, 10, 20 and 30 g of stock feed additive diet were mixed with 90, 80 and 70 g of commercial feed, respectively.

Experimental designs: Eighty, unvaccinated one-day-old, female Cobb 500 broiler chicks obtained from a commercial hatchery (Krungthai, Thailand) were randomly divided into 4 groups (20 chickens each); a

negative control group (0 g% PG), and 1, 2 and 3 g % PG groups (Pholdaeng and Pongsamart, 2010). Each group was randomly divided into 2 replicates. Each group of chicks was maintained in a separate unit and each replicate of 10 chicks was raised in a metallic cage at environmental temperature. All the chicks were fed *ad libitum*. All the chicks at 1, 7 and 14 days old were vaccinated with live Newcastle disease (ND) vaccine (Merial, France) by eye drops, inactivated ND vaccine (Fort Dodge®, Brazil) by subcutaneous injection and infectious bursal disease (IBD) vaccine (Merial, France) by oral drops, respectively. Blood was collected from the wing vein at 1, 7, 14, 28, 35 and 42 days of age and tested to measure blood cholesterol, heterophil:lymphocyte (H:L) ratio, hemagglutination inhibition (HI) titers for ND and ELISA titers for IBD. The antibody titers of IBD were determined using ELISA test kits (Synbiotic Corp, USA). Chickens at one day old and six weeks old in each group were weighed and the amount of feed intake was recorded for the calculation of weight gain. Delivery box-liners were swabbed for total bacteria and *Salmonella* suspected colony test. Feces were also collected to determine total bacteria count and *Salmonella* suspected colony count.

Heterophil : lymphocyte (H:L) ratio: Bloods were collected and mixed with EDTA as anticoagulant and carried out to measure the ratio of heterophil : lymphocyte (H:L). The bloods were smeared and stained with Wright-Giemsa (Hauptmanova et al., 2002), 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.

Isolation and determination of numbers of bacteria count

Preparation of samples: Fecal sampling was performed from each replicate. Two pooled, fecal samples were collected in each group. A sample collection technique was modified from Andreatti Filho et al. (2007). Samples of chicken feces were collected from 5 points of a fecal tray; 4 points at the corner and 1 point at the center of the tray and then pooled into 1 sample. One gram fecal samples were taken and mixed with 9 ml buffered peptone water (BPW) pH 7.5. The fecal suspension samples were used for determination of the total number of bacteria and *Salmonella* suspected colony counts.

Total plate count and *Salmonella* suspected colony count: The fecal suspended samples were diluted by ten fold serial dilution with BPW from 10⁻² to 10⁻¹³ dilution. Plate count agar medium (Merck KGaA, Darmstadt, Germany) was melted and poured into a petri dish, cooled to room temperature until solidified and then a 0.1 ml sample pipetted from the 10⁻² to 10⁻¹³ dilution was added into each petri dish, spreaded on the plate and incubated at 35°C for 24 hours. A plate that contained between 30 and 300 colonies was selected for counting (ISO, 2002). For the *Salmonella* suspected colony count, 1 ml of the fecal suspension

of each sample, obtained by mixing the fecal sample with BPW (1:9 w/v), was transferred into 9 ml of BPW; this suspension mixture was serially 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 (Merck KGaA, Darmstadt, Germany) and incubated for 24 hours at 37°C. Then, the black Salmonella suspected colonies were counted (Xiong et al., 1998).

Determination of cholesterol

Extraction of cholesterol from plasma: Cholesterol was extracted from 0.5 ml of chicken plasma at one day old and six weeks old with 0.5 ml cold methanol (Fisher Scientific, Loughborough, UK) and 2.5 ml hexane (Fisher Scientific, Loughborough, UK). The samples were vortexed and then centrifuged 80 x g at 4°C for 5 min. The hexane layer was removed and dried under N_2 and re-dissolved in a mobile phase composed of 1 ml acetonitrile : isopropanol (75 : 25, v/v) 1 ml (Fisher Scientific, Loughborough, UK) (Tippayakul et al., 2002) and transferred to a HPLC vial and then analyzed by HPLC instrument (Shimadzu Corp, Japan).

Extraction of cholesterol in breast muscles: Four chickens from each experimental group were randomly selected for euthanasia by cervical dislocation. The breast muscles of each chicken were collected and cholesterol extracted by the method of Folch et al. (1957). Briefly, one hundred grams of breast muscle were saponificated at 93°C with ethanol and potassium hydroxide. Later, the samples were extracted with distilled water and hexane until the layers separated. Then, the cholesterol was diluted in a hexane layer with N_2 gas and diluted with 1 ml of acetonitrile:isopropanol (75 : 25, v/v) and analyzed by the HPLC method (Shimadzu Corp, Japan).

Assay of cholesterol detection: The cholesterol content was assayed using the HPLC technique (Araki et al., 1990; Seta et al., 1990), a column symmetry C18 (3.9 x 150 mm, 5 μ m) was used and each of the 15 μ l samples was injected into the column. The mobile phase for HPLC was acetonitrile : 2-propanol (7 : 3), which had previously been filtered through a 0.45 μ m membrane filter before use. The column was eluted at a flow rate of 1.5 ml/min at ambient temperature and the UV detector was monitored at 210 nm.

Hemagglutination inhibition and ELISA test: The hemagglutination inhibition titers of ND were tested in a U-shaped, 96-well, microtiter plate (Corning, USA) as modified by Alexander (2000). In brief, a dilution series of sera was incubated with 4 hemagglutination units of ND virus, La Sota strain, at room temperature for 30 min. The hemagglutination unit was titrated before each assay. Thereafter, chicken erythrocytes were added and agglutination was monitored after incubation at room temperature for 45 min. The hemagglutination inhibition titer was defined as the reciprocal of the highest serum dilution completely inhibiting agglutination. The antibody titers of IBD were determined using ELISA test kits (Synbiotic Corp, USA). Briefly, ELISA plates were

coated with IBDV specific antibodies. Sample serum diluted 1/10 to 1/25 (w/v) in a dilution buffer was incubated in the coated wells. Unbound antigens were discarded at the end of the incubation period by washing with a washing buffer. The captured antigens were then revealed, as in an indirect ELISA, with a detection antibody, followed by an enzyme conjugate that bound to the detection antibody only followed by the enzyme substrate. Finally, optical densities, which paralleled the amount of captured IBDV antigens, were read with an ELISA reader (Etteradossin et al., 1997).

Statistical analysis: The antibody titers, cholesterol levels and weight gain were analyzed using ANOVA and Duncan's multiple range test with SPSS software (SPSS Inc, Chicago). Differences between groups were considered significant at $p < 0.05$.

Results

At forty two days old, the body weight gain (gram) (mean \pm SE) of chickens in groups 0, 1, 2 and 3 g% PG was 1,856.44 \pm 2.21, 1,873.67 \pm 10.29, 1,929.63 \pm 8.71 and 1,918.58 \pm 5.63, respectively. The feed intake (gram) (mean \pm SE) of chickens in groups 0, 1, 2 and 3 g% PG was 4,024.40 \pm 2.12, 3,890.29 \pm 2.28, 3,714.83 \pm 1.36 and 3,922.86 \pm 2.36, respectively. The results indicated that PG at a different ratio in a feed-supplement diet did not affect the chickens' weight gain in addition to total feed intake. The results revealed no significant difference between the group fed on the commercial feed without PG (control) and the groups fed on the commercial feed coated with PG 1, 2 and 3 g% PG.

At six weeks old, hemagglutination inhibition antibody titers against ND virus (Fig 1) and ELISA antibody titers against IBD virus (Fig 2) of the chickens in the 3 g% PG group were significantly different ($p < 0.05$). The chickens in the 3 g% PG group revealed higher titers against ND and IBD antibody than those of the other groups. At week 1 until week 5 the results revealed no significant difference between the group fed on the commercial feed without PG (control) and the groups fed on the commercial feed coated with PG 1, 2 and 3 g% PG.

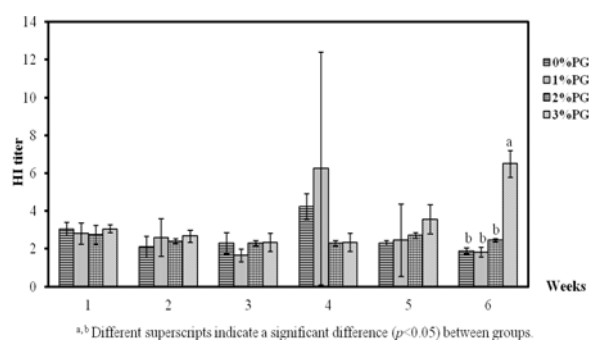


Figure 1 Hemagglutination inhibition antibody titers against Newcastle disease virus were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the geometric mean titer ($\log_2 \pm SD$) of twenty serum samples

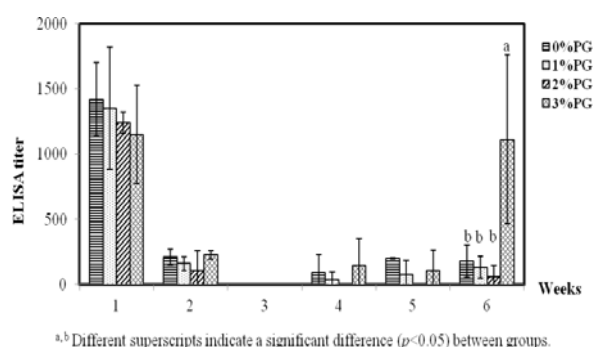


Figure 2 ELISA antibody titers against infectious bursal disease virus were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the arithmetic mean titer (mean±SD) of twenty serum samples.

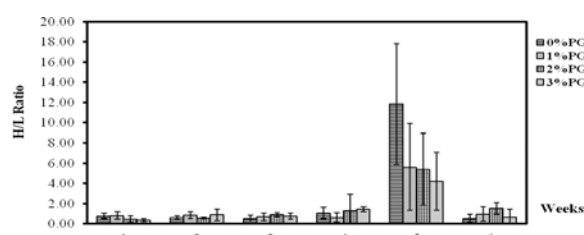


Figure 3 Heterophil : Lymphocyte ratio of white blood cell fraction in chickens were determined weekly in chickens receiving different concentrations of PG in feed. Each data set represents the arithmetic mean (mean±SD) of twenty serum samples.

The results of H:L ratio at the beginning until the end of the experiment revealed no significant difference between the group fed on the commercial feed without PG (control) and the groups fed on the commercial feed coated with PG 1, 2 and 3 g% PG (Fig 3). At five weeks old, the H:L ratio of chickens in each group was higher than the other weeks. Chickens in 0 g% PG group showed a higher H : L ratio than those of the other groups.

The swabs of delivery box-liners showed contamination of total bacteria and Salmonella suspected colony. The chickens in groups of 1, 2 and 3 g% PG exhibited lower colonies of total bacteria counts than the chickens in the control group (0 g% PG). At six weeks old, the total bacteria count of the chickens in groups 1, 2 and 3 g% PG was significantly reduced 81, 88 and 97%, respectively, compared to the group 0 g% PG ($p < 0.05$) (Table 1). The polysaccharide gel reduced the number of bacteria in chicken fecal samples and the increased PG in feed resulted in a decrease in the total bacteria count in chicken feces. The polysaccharide gel in the feed-supplement diet exhibited a killing effect on the bacteria in chicken feces. For the Salmonella suspected colony count, the amount of Salmonella suspected colonies in chicken feces was significantly lower in the groups fed on PG coated diets ($p < 0.05$) compared to that of the control group, at 5 and 6 weeks old (Table 2). The feed with PG showed zero Salmonella suspected colony count in 1 and 2 g% PG group at 5 weeks old. No Salmonella suspected colony was found in the chickens fed on a PG coated diet at 6 weeks old.

The average cholesterol levels in the plasma of chickens in groups 0, 1, 2 and 3 g% PG group at 1 day old and 6 weeks old are shown in Fig 4. The results revealed no significant difference between the group fed on commercial feed without PG (control) and the groups fed on commercial feed coated with PG 1, 2 and 3 g% PG ($p < 0.05$). The plasma cholesterol levels of the PG treated groups showed lower cholesterol levels compared to those of the control group. For the muscle cholesterol, the cholesterol level in the muscle of chickens in the 3 g% PG group was significantly reduced compared to that of the chickens in the control group (0 g% PG) ($p < 0.05$). The more the PG in feed the greater the cholesterol reduction in the chicken's muscle. Recalculated to a 100% cholesterol level in the muscle of the chickens in the control group (0 g% PG), the cholesterol level in the muscle of chickens in group 4 was reduced to 75.78% (Table 3).

Table 1 Total bacteria count (11 log₁₀ colony forming unit/ml) in fecal samples of chickens in each group/week determined weekly

Groups	Average total bacteria count (mean±SD)					
	1 week	2 week	3 week	4 week	5 week	6 week
0g% PG	171.25±14.43 ^{ab}	290.00±7.83 ^a	36.50±7.59	301.50±5.45 ^a	160.00±12.25 ^a	137.00±19.71 ^a
1g% PG	156.25±7.41 ^{bc}	155.00±26.04 ^c	45.00±4.97	120.50±56.04 ^b	32.00±15.43 ^b	26.00±18.24 ^c
2g% PG	126.50±2.12 ^c	128.00±16.43 ^c	87.50±62.04	111.00±15.14 ^b	11.00±6.22 ^c	16.25±4.65 ^c
3g% PG	200.75±31.91 ^a	236.50±42.61 ^b	36.75±31.08	55.75±15.84 ^c	4.75±2.22 ^c	4.50±1.73 ^c

^{a, b, c} Different superscripts indicate a significant difference ($p < 0.05$) between groups

Table 2 Salmonella colony count (2 log₁₀ colony forming unit/ml) in fecal samples of chickens in each group/week determined weekly

Groups	Average Salmonella suspected colony count (mean±SD)					
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
0g% PG	272.75±25.75 ^a	299.00±24.25 ^a	350.00±14.00 ^a	30.00±15.00	27.50±6.50 ^a	10.00±1.00 ^a
1g% PG	112.50±2.75 ^b	196.25±7.50 ^b	105.00±4.50 ^b	0	0 ^b	0 ^b
2g% PG	47.25±23.63 ^b	113.50±1.50 ^b	89.75±22.25 ^b	5.00±2.00	0 ^b	0 ^b
3g% PG	24.75±6.25 ^b	100.50±39.75 ^b	16.50±6.50 ^b	0	2.00±1.00 ^b	0 ^b

^{a, b, c} Different superscripts indicate a significant difference ($p < 0.05$) between groups

Table 3 Cholesterol levels in 6 chicken muscle samples in each group analyzed at 6 weeks old.

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

^{a, b} Different superscripts indicate a significant difference ($p < 0.05$) between groups

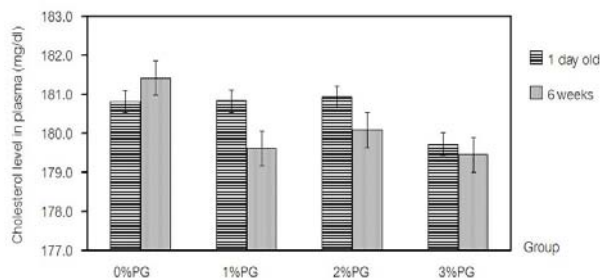


Figure 4 Cholesterol level in chicken plasma before and after being fed on different concentration of PG in feed for 6 weeks was determined. Each data set represents the arithmetic mean (mean±SD) of twenty serum samples.

Discussion

The body weight gain of the chickens in the control group was not significantly lower than those of the treatment groups. Therefore, the polysaccharide gel in the feed did not produce any adverse effects to the chickens. At six weeks old, the chickens in the group receiving 3 g% PG revealed the highest antibody titers against ND and IBD immunity and a more significant difference ($p < 0.05$) than the other groups (Fig 1 and 2). This is in accordance with Chansiripornchai et al. (2008) who reported that chickens fed on PG showed a better immunity against ND than non PG feeding group. Moreover, a preliminary study by Chansiripornchai et al. (2008) reported that adding PG to broiler chicken diet, as a feed additive, stimulated the humoral immune responses. Hokputsa et al. (2004) reported that the PG inhibited immunomodulating activity when estimated by complement fixation assay.

No significant difference was found in the H:L ratio between the experimental groups fed on PG and the control group (Fig 3). The Heterophil:Lymphocyte ratio is the ratio of heterophils on lymphocytes. An alteration of this value depends on the types and ages of animals and environment changes. When chickens are stressed, glucocorticoid hormone increases resulting in a decrease in lymphocytes (Puvadolpirod and Thaxton, 2000). In contrast to lymphocytes, when the heterophils increase, the H:L ratio also increases. The normal range of the H:L ratio of poultry is 0.33-0.5 (Gross,

1988). In this experiment, the H:L ratios were rather high, which might have been caused by the disturbance of the chickens by frequent serum collection and weighing in every week. Weighing chickens every week could cause stress, so glucocorticoid hormone increased and lymphocytes will decrease. At five weeks old, the H:L ratios of the chickens in all groups increased. This might have been caused by the noise from building work. Under stress condition, animals will release corticosteroids in the body, resulting in an increase in the mature neutrophil numbers (neutrophilia) with reductions in both the lymphocytes (lymphopenia) and eosinophils (eosinopenia) (Bush, 1991) or termed a stress leucogram. This is a transient change that occurs because there are shifts in the neutrophil from the marginal pool to the circulating pool. The stress leucogram may also be seen with corticosteroid administration (Barry, 1998). However, the chickens fed on 0 g% PG showed a higher H:L ratio than those chickens in the experimental groups. Thus, the chickens fed on a PG diet tended to have a better ability to resist stress than those chickens fed on a non PG diet.

At five and six weeks old, the total bacteria count and Salmonella suspected colony count in the chicken feces of the control group were significantly higher than those of the experimental groups ($p < 0.05$) (Table 1 and 2). Polysaccharide gel reduced Salmonella suspected colonies in chicken feces even at lower amounts of PG (1 g% PG). The polysaccharide gel has an antibacterial activity that restrains both Gram positive and Gram negative bacteria (Lipipun et al., 2002; Pholdaeng and Pongsamart, 2010). Therefore, the polysaccharide gel could reduce bacteria in chicken feces and the higher PG in the diet showed a better reduction in the total bacteria count in the feces. Polysaccharide gel is not absorbed through the stomach (Tippayakul et al., 2002), so it passes through the small intestine where it is firstly infected with Salmonella (Bangtrakulnonth, 2002). In addition, the physical properties of PG include an 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). Polysaccharide gel has an intrinsic viscosity and adhesive properties due to the electronegativity and branch chain neutral sugars of the pectic polysaccharide, which probably adhesively bind on the cell's outer surface (Lipipun et al., 2002; Pongsamart et al., 2005). Moreover, polysaccharide gel may cause adhesion interference to the cell's normal function and alter the membrane permeability (Tsai and Su, 1999).

No significant difference in cholesterol in the chickens' plasma between the control and the experimental groups was found (Fig 4). However, the chickens fed commercial feed with PG tended to have a lower cholesterol level in the plasma than the chickens fed on normal feed. This result agrees with

Chansiripornchai et al. (2008) who discovered that mixing PG in the chicken diet could reduce the cholesterol level in serum. Moreover, the cholesterol levels in the muscle of the chickens fed on 3 g% PG was significantly lower than those of the chickens in the control group ($p < 0.05$) (Table 3). A preliminary study indicated that the level of cholesterol in muscle reduced when PG was added in feed as determined by a colorimetric method (Chansiripornchai et al., 2008). This method is based on the determination of cholestenone after enzymatic cleavage of the cholesterol ester, conversion of cholesterol, and the consequent formation of a red dyestuff after the reaction of 4-aminophenazone with phenol. The color intensity is directly proportional to the concentration of cholesterol and is determined photometrically (Allain et al., 1974).

Although the analysis method was different in this case, the result was the same. It was confirmed that PG in the chicken diet could potentially reduce the cholesterol in the chickens' muscle. Polysaccharide gel can well confine liquid such as cholesterol and fatty acid by absorbing lipids and cholesterol in the alimentary canal resulting in less confinement of lipids and cholesterol in chickens (Tippayakul et al., 2002). 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. The increased 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 a 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 the chickens fed a diet with PG was lower than that of the chickens fed a diet without PG. Therefore, polysaccharide gel can reduce cholesterol in chickens. Polysaccharide gel may be used as a feed-supplement for broiler chickens in order to reduce contaminated bacteria in the gastrointestinal tract and also decrease cholesterol in chickens' muscle. In the future, polysaccharide gel in chickens may promote human health by reducing cholesterol level and cardiovascular diseases.

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