Efficacy of dietary chitosan on growth performance, haematological parameters and gut function in broilers

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**ABSTRACT**

Percentages of antimicrobial resistance to Salmonella and \textit{Escherichia coli} in poultry products were high in Thailand, thus, alternative to antibiotic growth promoter is required. The objective of this experiment aimed to examine the effect of dietary chitosan in broilers on blood and intestinal changes. Total of 392, day-old male Ross 308 broilers were allotted into four groups. Broilers in the control group (group 1) received commercial corn-soybean meal-based basal diet. Chicks in group 2 received basal diet supplemented with 200 ppm amoxicillin. Chitosan was added into the basal diet at 1 and 2 g/kg in groups 3 and 4, respectively. Data on growth performance as well as blood and digesta samples were collected on d 21 and 39. The results showed that 2 g/kg dietary chitosan significantly improved feed conversion ratio (FCR) during d 22-39 and 1-39 (\(p < .05\)). This level of chitosan also increased the ratio of villus height (VH) and crypt depth (CD) (\(p < .05\)) while decreased the CD (\(p < .05\)). The supplementation of 1 and 2 g/kg chitosan in diet tended to reduce the ammonia-nitrogen in colonic digesta. Furthermore, both levels of chitosan enhanced number of \textit{Bacillus} spp. while decreased number of \textit{E. coli} (\(p < .001\)) leading to increased ratio of \textit{Lactobacillus} and \textit{E. coli} (\(p < .001\)). There were no significant effects on the heterophil (H): lymphocyte (L) ratio and coefficient of apparent ileal digestibility. In conclusion, the supplementation of 2 g/kg chitosan in broiler diets could be used as an alternative additive to antibiotic with its improvement on gut function.

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**Introduction**

Chitosan is originated from chitin, a polysaccharide formed by N-acetyl-D glucosamine units found in insects, marine diatoms, algae, fungi and crustacea, by deacetylation, demineralisation, deproteinization and decolouration (Keser et al. 2011). Several studies have been conducted on chitosan as an animal feed supplement but they have given variable results. It was reported previously that dietary supplementation of chitosan could improve animal growth performance (Xu et al. 2014). Dietary chitosan at low concentrations of 0.5–1 g/kg tended to enhance growth rate due to increased nitrogen utilisation and amino acid digestibility (Shi et al. 2005). In addition, body weight gain (BWG) of broilers fed with supplementation of chitosan at 0.6 g/kg was better than that of the control group (Khammadalai et al. 2008). However, another study reported a negative impact of chitosan on growth performance (Zhang et al. 2008). Effects of dietary chitosan on the growth performance in broiler chickens are still controversial.

Chitosan has been regarded as an additive with multifunctional activities for instances, chitosan acted as antimicrobial material against foodborne pathogens (Kong et al. 2010). The addition of 0.2% chitosan in diet could significantly decrease colonisation of \textit{Salmonella} Typhimurium in vitro and in broilers (Menconi et al. 2014). Chitosan statistically reduced levels of total cholesterol, low-density lipoprotein cholesterol in plasma and total triglyceride in rat liver (Xu et al. 2007). The addition of 10% w/w chitosan to the broiler litter reduced total and organic nitrogen loss as well as concentrations of ammonia-producing bacteria and fungi compared to the control litter group without chitosan (Cook et al. 2011). There has been no report on the relationship between dietary chitosan and haematological parameters of broilers in the tropical climate. The percentages of antimicrobial resistance to bacteria in livestock including broilers were...
high in Thailand (Chuanchuen 2013). Therefore, possible alternative additive to replace antibiotic in improving growth performance and gut function but reducing ammonia volatilisation in broiler production was needed (Attia et al. 2011). This study aimed to investigate the effects of two levels of dietary chitosan on growth performance, apparent ileal digestibility, jejunal morphology, caecal microbiota, colonic ammonia concentration, haematological parameters and serum lipids in broilers.

**Materials and methods**

The experimental protocol followed relevant guidelines and regulations on the use of animals for scientific purposes and was certified by the Chulalongkorn University Animal Care and Use Committee (permission No. 1531041).

**Birds, diets and environment**

The chicks were vaccinated with the Newcastle plus Infectious Bronchitis vaccine at the hatchery. One-day-old male Ross 308 broilers (n = 392) were allocated into 4 groups of 7 replicates (14 chicks each) in a complete randomised design. Group 1 (T1) was the control group; chicks were fed on commercial basal diet composed of corn-soybean meal as a major ingredient (Table 1), while chicks in group 2 (T2) received 200 ppm amoxicillin in the basal diet. Groups 3 (T3) and 4 (T4) were supplemented with chitosan 1 and 2 g/kg in the basal diet, respectively. Feed and water were provided ad libitum. Proximate analyses of diets were performed according to AOAC (2012) methods. The basal diet met or exceeded the nutrient requirements recommended by NRC (1994). The chitosan used in this experiment was provided by Sea fresh Industry Public Co., Ltd. (Bangkok, Thailand). Its degree of deacetylation was determined to be 85% with molecular weight 500,000 dalton and particle size 60 mesh. Chicks in all groups were raised under the same condition in an open house at a density of 9.3 birds/m². The floor was covered with a 10 cm height of rice hull litter. The experiment was conducted from July to August. Average temperature and relative humidity during the experiment were 31.1 ± 2.2 °C and 66.8 ± 9.6%, respectively.

**Sampling and measurements**

**Growth performance**

Feed intake was recorded daily during the experimental period. Broilers were individually weighed on d 1, 21 and 39 of the trial. Growth performance was determined in terms of BWG, feed conversion ratio (FCR) and mortality rate for all groups on d 21 and 39.

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**Table 1.** Ingredient composition and chemical analysis of basal diet (g/kg DM unless classified otherwise).

| Item                      | Starter (d 1–21) | Grower (d 22–32) | Finisher (d 33–39) |
|---------------------------|------------------|------------------|-------------------|
| Corn                      | 581.0            | 614.4            | 665.1             |
| Oil                       | 13.0             | 27.9             | 28.4              |
| Soybean meal              | 360.7            | 316.5            | 268.9             |
| Calcium carbonate         | 14.3             | 13.1             | 11.9              |
| MCP-22a                   | 17.9             | 16.0             | 14.6              |
| Salt                      | 3.1              | 3.1              | 3.0               |
| DL methionine             | 3.4              | 3.0              | 2.7               |
| L-lysine                  | 2.5              | 2.2              | 2.2               |
| Threonine                 | 0.9              | 0.7              | 0.4               |
| Choline chloride (60%)    | 0.8              | 0.9              | 0.9               |
| Antioxidant               | 0.1              | 0.1              | 0.1               |
| Anticoccidial             | 0.5              | 0.5              | ~                 |
| Vitamin-mineral premix    | 1.8              | 1.8              | 1.8               |

Chemical analyses

| Gross energy, MJ/kg       | 16.3             | 17.0             | 16.7              |
| Crude protein             | 221.8            | 205.0            | 183.3             |
| Crude fat                 | 40.8             | 54.5             | 55.8              |
| Crude fibre               | 17.3             | 23.8             | 21.5              |
| Ash                       | 76.8             | 53.0             | 65.1              |
| Calcium                   | 5.9              | 6.0              | 6.4               |
| Phosphorus                | 4.8              | 5.2              | 4.4               |

*a* Monocalcium phosphate 22%.

*b* BHT (butyl-hydroxytoluene), BHA (butyl-hydroxyanisol) and ethoxyquin.

*c* Salinomycin.

*Premix provided the following per kg of diet: Vitamin A 4,800,000 IU, Vitamin D₃ 1,200,000 IU, Vitamin E 6000 IU, Vitamin K₃ 0.6 g, Vitamin B₁ 0.6 g, Vitamin B₂ 2.2 g, Vitamin B₆ 0.8 g, Vitamin B₁₂ 0.004 g, Nicotinic acid 10 g, Pantothenic acid 4.8 g, Folic acid 0.2 g, Biotin 0.048 g, Manganese (Mn) 32 g, Zinc (Zn) 24 g, Iron (Fe) 16 g, Copper (Cu) 3.2 g, Iodine (I) 0.2 g, Cobalt (Co) 0.04 g, Selenium (Se) 0.04 g, Antioxidant 0.05 g.
**Biological assessments**

**Blood samples**
At the age of 21 and 39 d, two chicks per replicate were randomly selected and then collected blood samples from wing vein or jugular vein using sterile needles. Whole blood samples (using EDTA as anticoagulant) were analysed for the complete blood count (CBC) and the ratio of heterophil and lymphocyte (H/L). Whole clotted blood was centrifuged to separate serum for determining cholesterol and triglyceride concentrations using commercial colorimetric assay kits.

**Microbiological determination**
After blood collection, both chicks in each replicate were euthanatised by an intracardial injection of sodium pentobarbital. Jejunal, ileal, caecal and colonic digesta samples were collected and kept in the freezer. Caecal digesta samples were determined for *Escherichia coli* and *Salmonella* spp. using guideline following to ISO 7251:2005 and ISO 6579:1993E.

**Jejunal morphology**
The histological measurement of jejunum was carried out as the procedures suggested by Awad et al. (2009) and Tsirtsikos et al. (2012) on two chicks per replicate. In brief, the jejunum located between bile duct entry and Meckel’s diverticulum approximately 2 cm length was collected and immersed in 4% buffered formalin for 48 h and then kept in 50% ethyl alcohol. Four cross-sections for each jejunal sample were prepared after staining with haematoxylin and eosiin using standard paraffin-embedding procedures. Ten intact, well-oriented crypt-villus units were chosen in each triplicate per jejunal cross-section. A portion of villus height (VH) was indicated from the tip of villus to villus crypt junction. The depth of the invagination between adjoining villi was identified as crypt depth (CD). All morphological estimations were performed in 10-μm increments using an image processing and analytical system with Image-PRO®PLUS 6.0 (Media Cybernetics Inc., Bethesda, MD)

**Apparent ileal digestibility**
All diets contained 2% Celite™ (Lompoc, CA) as a source of an indigestible marker, acid insoluble ash (AIA), on d 16–20 and 34–38. Ileal digesta samples from two chicks in each replicate were collected, pooled together and stored at −20°C. AIA in feed and digesta samples were analysed according to Angkanaporn et al. (1996). The coefficient of apparent ileal digestibility (CAID) of the nutrients was then calculated from the equation: CAID = 1 – [(ileal nutrient/ ileal AIA)/(diet nutrient/diet AIA)]

**Colonic ammonia-nitrogen concentrations**
On d 21 and 39, colonic digesta samples from 2 broilers per replicate were collected in plastic bottles and then kept in the freezer. These samples were determined for ammonia-nitrogen concentrations using phenol-hypochlorite method (Strickland and Parsons 1972)

**Statistical analyses**
The effect of the feeding treatments on each experimental parameter was analysed using one-way ANOVA on the data set comprising 7 replicates per treatment. The number of subjects in each replicate varied according to the age and the assessment as previously described and specified in tables. When a significant ($p < .05$) $F$ statistic was noted, treatment means were separated by Duncan’s new multiple range tests.

**Results**

**Growth performance**
As shown in Table 2, the ADG on d 1–21 and FCR of chicks in all periods (d 1–21, 22–39, 1–39) in group 2 were significantly better ($p < .05$) than those of group 3. However, there was no significant difference between the control group (group 1) and the chitosan groups (groups 3 and 4). FCRs of birds in group 2 were not different from those of group 4 in all periods. Live weights of birds in group 2 on d 21 were significantly greater ($p < .05$) than those of group 3 while no significant difference was found between the control group and both chitosan groups on d 21. There was no significant difference of live weight among groups on d 39. The mortality rates were not different among groups during d 1–21. Group 3 had the highest mortality rate during d 22–39 compared with other groups ($p < .05$).

**Haematological parameters**
Most of haematological parameters in all treatments were not different except the white blood cell (WBC) count (Table 3). Chicksens in group 4 had higher WBC count than those of groups 1 and 2 ($p < .05$) but it was not different from group 3. There was no
significant difference in H/L ratio of chickens among groups on d 39.

Serum cholesterol and triglyceride

Effects of chitosan supplementation on serum cholesterol and triglyceride of broiler chickens on d 39 are shown in Table 3. There was no effect on serum cholesterol and triglyceride with chitosan supplementation groups compared with groups 1 and 2.

Microbial populations

No significant difference was found among the four groups in the beneficial bacteria (e.g. *Bacillus* spp., lactic acid bacteria) and pathogenic bacteria (e.g. *E. coli*, *Salmonella* spp.) as well as the ratio of *Lactobacillus* and *E. coli* on d 21 (Table 4). On d 39, chickens in group 3 tended to increase the number of *Bacillus* spp. when compared with the control group although there were not different among groups 2, 3 and 4.

### Table 2. Growth performance of broilers in the various treatments in each period of the study (7 replicates per treatment).

| Treatment | T1       | T2       | T3       | T4       | SEM    | p-Value |
|-----------|----------|----------|----------|----------|--------|---------|
|           | ADG, g/bird/d | Feed intake, g/bird/d | Feed conversion ratio, FCR | Live weight, g | Mortality rate, % |         |
| d 1–21 (14 birds per replicate) | 42.9<sup>a</sup> | 44.8<sup>b</sup> | 40.9<sup>b</sup> | 42.8<sup>b</sup> | 0.5 | .02 |
|           | 59.0 | 59.2 | 58.6 | 58.0 | 0.3 | .52 |
|           | 1.4<sup>b</sup> | 1.3<sup>b</sup> | 1.4<sup>a</sup> | 1.4<sup>b</sup> | 0.01 | .05 |
|           | 944<sup>b</sup> | 982<sup>a</sup> | 901<sup>b</sup> | 940<sup>b</sup> | 9.5 | .02 |
|           | 1.4 | 0.0 | 2.9 | 0.0 | 0.6 | .27 |
| d 22–39 (12 birds per replicate) | 84.7 | 91.4 | 83.3 | 90.2 | 1.7 | .24 |
|           | 184.2 | 187.2 | 196.2 | 183.9 | 2.5 | .29 |
|           | 2.2<sup>b</sup> | 2.1<sup>b</sup> | 2.4<sup>a</sup> | 2.0<sup>b</sup> | 0.1 | .04 |
|           | 1.4<sup>b</sup> | 0.0<sup>b</sup> | 8.6<sup>a</sup> | 2.8<sup>b</sup> | 1.0 | .01 |
| d 1–39 | 62.2<sup>a</sup> | 66.3<sup>a</sup> | 60.5<sup>b</sup> | 64.6<sup>b</sup> | 0.9 | .05 |
|           | 116.8 | 118.3 | 122.1 | 116.1 | 1.2 | .29 |
|           | 1.9<sup>a</sup> | 1.8<sup>b</sup> | 2.0<sup>a</sup> | 1.8<sup>b</sup> | 0.03 | .03 |
|           | 2468 | 2627 | 2400 | 2564 | 36 | .12 |
|           | 2.9<sup>b</sup> | 0.0<sup>b</sup> | 11.4<sup>a</sup> | 2.9<sup>b</sup> | 1.3 | .005 |

### Table 3. Complete blood count and serum lipids of broilers in each treatment on d 39 (2 chicks per replicate, 7 replicates per treatment).

| Treatment | T1       | T2       | T3       | T4       | SEM    | p-Value |
|-----------|----------|----------|----------|----------|--------|---------|
|           | RBC x 10<sup>6</sup> cells/µL | Haemoglobin, g% | Haematocrit, % | WBC, cells/µL | Heterophil, % | Basophil, % | Eosinophil, % | Lymphocyte, % | Monocyte, % | Heterophil/lymphocyte (H/L) ratio | Cholesterol, mg/dL | Triglyceride, mg/dL |
|           | 2.1 | 2.1 | 2.1 | 2.3 | 0.1 | .32 |
|           | 8.2 | 8.1 | 8.4 | 8.4 | 0.2 | .92 |
|           | 24.6 | 23.9 | 25.0 | 24.7 | 0.6 | .91 |
|           | 8045<sup>b</sup> | 9570<sup>b</sup> | 10,622<sup>b</sup> | 13,624<sup>a</sup> | 74 | .04 |
|           | 67 | 67 | 64 | 66 | 0.7 | .49 |
|           | 0 | 0 | 0.7 | 0.3 | 0.1 | .08 |
|           | 31 | 30 | 31 | 30 | 0.7 | .95 |
|           | 2 | 3 | 4.3 | 3.7 | 0.4 | .31 |
|           | 2.2 | 2.3 | 2.2 | 2.3 | 0.1 | .91 |
|           | 122 | 124 | 127 | 122 | 3 | .94 |
|           | 60 | 75 | 78 | 81 | 4 | .31 |

### Table 4. Caecal microbial populations (log<sub>10</sub> cfu/g) of broilers in various treatments (7 replicates per treatment).

| Treatment | T1       | T2       | T3       | T4       | SEM    | p-Value |
|-----------|----------|----------|----------|----------|--------|---------|
|           | Bacillus spp. | Lactic acid bacteria | *E. coli* | Lactobacillus: *E. coli* ratio | | |
| d 21<sup>a</sup> | 5.5 | 5.9 | 5.6 | 5.6 | 0.06 | .06 |
|           | 7.8 | 7.2 | 7.2 | 7.4 | 0.12 | .27 |
|           | 7.3 | 7.4 | 7.4 | 7.2 | 0.15 | .96 |
|           | 1.08 | 0.98 | 0.98 | 1.04 | 0.02 | .56 |
| d 39<sup>bc</sup> | 4.5<sup>b</sup> | 4.6<sup>b</sup> | 4.7<sup>b</sup> | 4.6<sup>b</sup> | 0.03 | .05 |
|           | 7.1<sup>a</sup> | 6.8<sup>a</sup> | 6.9<sup>a</sup> | 7.1<sup>a</sup> | 0.04 | <.001 |
|           | 6.7<sup>a</sup> | 4.8<sup>b</sup> | 1.8<sup>c</sup> | 1.7<sup>c</sup> | 0.57 | <.001 |
|           | 1.08<sup>a</sup> | 1.51<sup>b</sup> | 3.79<sup>a</sup> | 4.18<sup>a</sup> | 0.27 | <.001 |

<sup>a,b,c</sup>Means in the same row with unlike superscripts were significantly different (p < .05).
Apparent ileal digestibility

The CAID of protein and fat were not different among groups on d 21 and 39. However, group 2 had higher CAID of protein than group 3 ($p = .05$) but it was not different from groups 1 and 4 on d 39 (Table 5).

Jejunal morphology

From Table 6, there were no significant differences in VH of mid jejunum among groups on d 21 and 39. The crypt depth (CD) in group 2 was lowest on d 21 compared with other groups ($p < .05$) but there were not different among groups 1, 3 and 4. The control group had the deepest crypt depth ($p < .05$) while no differences were found in groups 2, 3 and 4 on d 39. VH/CD on d 21 in group 2 was higher than that of group 4 ($p < .05$) but no differences were found in groups 1, 3 and 4. In addition, group 4 had VH/CD greater than the control group on d 39 ($p < .05$).

Colonic ammonia-nitrogen concentrations

The broiler chickens with two levels of chitosan supplementation (groups 3 and 4) could reduce colonic ammonia-nitrogen concentrations on d 21 compared with the control group ($p < .05$). The addition of chitosan 1 g/kg diet (group 3) decreased ammonia-nitrogen concentrations on d 39 compared with the amoxicillin group (group 2) ($p = .05$) while it was not different from groups 1 and 4 (Table 7).

Discussion

The addition of 2 g/kg chitosan in diet tended to promote FCR as similar to amoxicillin addition. Broiler chickens supplemented with either 0.5 or 1.0 g/kg chitosan in diet tended to have better growth and feed conversion efficiency since chitosan probably played a vital role in regulating intestinal microflora including enhancing digestion and absorption of protein (Shi et al. 2005). However, male Marshall Chunky broilers fed with 0.6 g/kg chitosan for 7 weeks of age could improve BWG and feed intake but had no difference in feed efficiency (Khambuailai et al. 2009). It should be noted that the results of growth performance in broiler chickens from several studies were different, possibly owing to a variety of chitosan characteristics such as concentration, degree of acetylation and molecular weight (Goy et al. 2009; Khambualai et al. 2009). The highest mortality rate of chicks in group 3 during finisher period may be due to high temperature (30°C) and relative humidity (80%) in the late afternoon on d 35. The broiler chickens were raised in the open house therefore there may be some areas with poor ventilation.

Broiler chickens fed with 1 and 2 g/kg chitosan increased WBC numbers. It is in accordance with Meng et al. (2010) who found that laying hens fed with

Table 5. The coefficients of apparent ileal digestibility (CAID) of protein and fat (7 replicates per treatment).

| Treatment | T1 | T2 | T3 | T4 | SEM | p-Value |
|-----------|----|----|----|----|-----|--------|
| d 21      |    |    |    |    |     |        |
| Protein   | 0.91 | 0.87 | 0.86 | 0.86 | 0.02 | .098   |
| Protein   | 0.84ab | 0.85a | 0.71b | 0.78ab | 0.08 | .050   |
| Fat       | 0.82 | 0.81 | 0.62 | 0.79 | 0.19 | .738   |
| d 39      |    |    |    |    |     |        |
| Protein   | 0.84ab | 0.85a | 0.71b | 0.78ab | 0.08 | .050   |
| Fat       | 0.82 | 0.81 | 0.62 | 0.79 | 0.19 | .738   |

*Fat not determined at d 21.

*a,b Means in the same row with unlike superscripts were significantly different ($p < .05$).

Table 6. Villus height (VH), crypt depth (CD) and VH/CD of jejunal morphology (7 replicates per treatment).

| Treatment | T1 | T2 | T3 | T4 | SEM | p-Value |
|-----------|----|----|----|----|-----|--------|
| d 21      |    |    |    |    |     |        |
| Villus height, μm  | 803.3 | 692.9 | 659.8 | 778.9 | 29.7 | .27    |
| Crypt depth, μm     | 115.1a | 86.3b | 101.8ab | 124.6a | 4.7  | .02    |
| Villus height/crypt depth | 7.1ab | 8.0a | 6.6ab | 6.4b | 0.3  | .045   |
| d 39      |    |    |    |    |     |        |
| Villus height, μm  | 1109.1 | 981.1 | 965.8 | 1057.4 | 27.2 | .21    |
| Crypt depth, μm     | 179.2a | 150.0b | 138.7b | 139.3b | 5.7  | .03    |
| Villus height/crypt depth | 6.3ab | 6.9ab | 7.0ab | 7.6a | 0.3  | .04    |

*a,b Means in the same row with unlike superscripts were significantly different ($p < .05$).

Table 7. Ammonia-nitrogen concentrations in colonic contents of chicks on d 21 and d 39 of age (7 replicates per treatment).

| Treatment | NH₃-N concentration, mg/L | T1 | T2 | T3 | T4 | SEM | p-Value |
|-----------|---------------------------|----|----|----|----|-----|--------|
| d 21      |                          | 28.9 | 22.4 | 17.1 | 17.3 | 1.6 | .02    |
| d 39      |                          | 23.2 | 25.3 | 18.0 | 22.2 | 1.0 | .05    |

*a,b Means in the same row with unlike superscripts were significantly different ($p < .05$).
chito-oligosaccharide (COS) at 0.4% level had higher WBC number than other groups at 42 d of age. The H/L ratio, an indicator of stress, was not different in all groups but its value in each group was higher than the average range of 0.23 ± 0.003 in laying hens kept in the conventional floor pens (Singh et al. 2009). This result implied that broiler chickens were faced with heat stress problem.

The chickens fed with 1 and 2 g/kg chitosan in diet significantly decreased the number of E. coli but increased the ratio of Lactobacillus and E. coli. The current study corresponded well with the results of several researchers. For instance, chitosan (250 ppm at pH 5.3) appeared to adhere to the outer membrane of gram-negative bacteria (i.e. E. coli), thus losing the barrier function (Helander et al. 2001). The use of 10% and 15% chitin from shrimp shell in broiler diet could decrease E. coli and Salmonella spp. in the small intestine (Chitsatchapong 2009). Moreover, the concentration of 10% dietary chitin from shrimp shell also decreased Salmonella spp. in the digesta but increased Lactobacillus spp. in the intestinal digesta of broilers. Goy et al. (2009) proposed three antibacterial mechanisms: first, the ionic surface interaction, leading to cell wall leakage. Gram-negative bacteria (e.g. Salmonella spp., E. coli) seemed to be highly sensitive to chitosan compared to gram-positive bacteria (e.g. Bacillus spp., Lactobacillus spp.). It is consistent with these results because broiler chickens received 1 and 2 g/kg chitosan in diet were able to reduce the number of E. coli while increase the number of Bacillus spp. compared with the control group. Second, there was an inhibition of the mRNA and protein synthesis by passing chitosan through the nuclei of the microorganisms. Third, the formation of an external barrier, chelating metals resulted in suppressing essential nutrients to microbial growth. In general, chitosan that has lower molecular weight and degree of acetylation is able to efficiently reduce microorganism growth and multiplication.

The CAID of protein and fat in chitosan groups tended to decline on d 39. This is probably due to increased gastric and duodenal viscosities, binding of duodenal micelle components and delayed gastric emptying. In addition, it could be due to ineffective enzyme digestion of chitosan. However, the use of 0.5–1.0 g/kg dietary chitosan improved the utilisation of nitrogen compared with the control group (Shi et al. 2005).

The change in intestinal morphology of this study was in agreement with the previous results. No difference was found in the intestinal VH and villus area between the control group and the 0.6 g/kg dietary chitosan in broiler chickens (Khammadal 2009). Weaned pigs fed with 150 mg/kg short-chain chitosan could increase the VH/CD of jejunum on d 56 compared with the control group but it was not different from the 110 mg/kg dietary lincomycin group (Suthongsa et al. 2012). However, the increasing levels of chitosan increased quadratically the VH of jejunum and ileum. Meanwhile, chitosan increased quadratically VH/CD in all parts of small intestine and decreased quadratically the ileal crypt depth in weaned pigs (Xu et al. 2013). The VH/CD is a useful tool in the evaluation of nutrient absorption potential and the epithelial cell turnover in the small intestine (de Verdal et al. 2010). For the present results, it indicated that the dietary chitosan at two levels was positively attributed to the epithelial cell turnover similar to 200 ppm amoxicillin addition in diet on d 39. In agreement with the improved FCR and reduced E. coli number in caecum, the 2 g/kg dietary chitosan resulted in an improvement of VH/CD in jejunum on d 39. In general, coliform bacteria can impair the normal morphology of small intestinal mucosa but chitosan provided a useful environment for the enterocyte proliferation, forbidding intestinal atrophy (Han et al. 2012).

Chitosan had no effect on serum cholesterol and triglyceride of broilers in this trial. On the contrary, broilers fed on dietary chitosan showed the reduction of plasma triglyceride concentrations on d 11 and plasma cholesterol concentrations on d 11 and 19 (Razdan and Pettersson 1994). It was noted that the levels of chitosan at 1 and 2 g/kg of diets in this study were lower than those of the previous studies, that is, 30 g of chitosan/kg of broiler diets (Razdan and Pettersson 1994) as well as dietary 50 g/kg chitosan with low viscosity in broiler chickens (Kobayashi et al. 2002). Therefore, the chitosan at low level was not able to reduce the concentration of serum cholesterol and triglyceride in broilers.

From the present result, the dietary chitosan at 1 and 2 g/kg could reduce ammonia-nitrogen concentrations in broilers to some extent. Chitsatchapong (2009) reported that broilers fed with dietary chitin in the forms of shrimp shell meal (5%, 10%, 15%, 20%) and purified chitin (1.07%, 2.26%, 3.34%, 4.53%) were able to decrease ammonia in manure on d 21 while they fed with dietary purified chitin at all levels and 20% shrimp shell meal chitin also decreased ammonia in manure on d 28. Moreover, broilers supplemented with shrimp shell meal (5%, 10% and 15%) and purified chitin (1.07%, 4.53%) in diet could reduce ammonia in digesta. Ammonia was originated from proteins and their derivatives by several processes such as...
proteolysis, peptide degradation, deamination and deamidation. Bacteria play a vital role in ammonia producers such as gram-negative anaerobic and aerobic rods, clostridia and Bacillus spp. The dominant species of bacteria produced ammonia in colon are gram-negative anaerobes, and all the clostridia, mostly Clostridium perfringens including bacteria-producing urease and uricase (Cook et al. 2011). It is possible that chitosan was able to reduce these bacteria, leading to decreased ammonia-nitrogen in large intestine and manure, or the enhancement of ammonia absorption from large intestine. Chitsatchapong (2009) also proposed that chitosan has its polycationic and adsorbent property, thereby combining with ammonia and converting into nitrate in the intestine. It is noted that ammonia concentrations in the broiler house may differ from that in the colon. It is due to several factors such as temperature, relative humidity, animal density and the rate of air movement (Talukder et al. 2010).

Conclusions

From the overall results, it is concluded that chitosan at 2g/kg could be used as an effective alternative to antibiotics in broiler diets with an improvement of gut function and changes in microbial populations.

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