Blood Biochemical Indices and Productivity of Broilers on Diet Supplemented with Mannan Oligosaccharide, Baker Yeast, or Combined Baker Yeast and Noni Leaves Extracts

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Abstract: A research was carried out to study the inclusion of MOS, inactivated baker yeast or combined baker yeast and Noni leaves extracts into corn-mungbean basal diet on broilers blood biochemical indices and productivity. Lohman MB-202 day old chicks were randomly assigned into four treatment diets i.e. 1) commercial/control diet (Dc), 2) basal diet plus MOS (Dm), (3) basal diet plus inactivated baker yeast (Dy), (4) basal diet plus inactivated baker yeast and Noni leaves extracts (Dyl). Treatment diets and water were given ad libitum. Whole blood were sampled on day 21 for determination of hemoglobin and haematocrit, Serum Glutamic Pyruvic Transaminases, Glutamic Oxaloacetic Transaminases, creatinine, lipid profile, alkaline phosphatase and lactic acid. Relative organs weight, abdominal fat, productivity were also determined. Feed intake, body weight and feed conversion of broilers fed Dc, Dm, Dyl were the same but they were lower than control diet (p<0.05). Serum triglyceride, GPT, creatinine, cholesterol, VLDL+LDLChol and HDLChol were significantly affected by the diets (p<0.05). No effect was found for other serum biochemical indices. For relative internal organs weight, a significant effect was found for heart, liver, ileum, duodenum and pancreas. These results were discussed in terms of the possible underlying mechanism. Our results here provide evidence that MOS, inactivated baker yeast alone or its combination with Noni leave extract could produce low serum TG, total cholesterol, VLDL+LDLChol, HDLChol and abdominal fat in broilers fed corn-mungbean basal diet with no negative effect on other serum biochemical indices.

Key words: MOS, baker yeast, Noni leaves extracts, mungbean, biochemical indices

INTRODUCTION

The ban of feed antibiotic use worldwide and its adoption in some European countries do not affect its practical use in broiler production in Indonesia. This is due to the fact that antibiotic in feed and medication program can effectively prevent bacterial infection and maintain good animal performance to prevent environmental stress from hot and humid climate. However, antibiotic use in broiler production has been known to have negative effect i.e. the emergence of antibiotic resistance microbes and residual antibiotics in animal product which can compromise the health of consumers. In addition, the negative effects on biochemical indices such as increase blood cholesterol. Serum Glutamic Oxaloacetic Transaminase (SGOT) and abdominal fat are often overlooked as broilers are raised for short period of time (± 30-35 days) (Muwani and Bayuardhi, 2007). However, broiler industries have quickly responded and turned to natural additives such as pre-, pro- and phyto-biotics, enzymes, etc to maintain performance. Prebiotic such as MOS and yeast as well as phytobiotics have been shown to improve gut health and immunity in broilers (Gao et al., 2008; Windisch et al., 2008). They, therefore alone or in combination could be used to maintain gut condition which is important in digestion, absorption and subsequent metabolism of nutrients.

Broiler diet has also been shown to play important role in modulating body system under management without antibiotics. Feed ingredients in the diet can provide not only macro and micro nutrients but also phytoneutrins such as carotenoids and polyphenolic substance which are beneficial to the birds (Awika et al., 2000; Murwani, 2006; Murwani and Murtini, 2009). Several studies which assessed the use of such feed ingredients in a diet combined with phytobiotics tea mistletoe extract in the absence of in-feed antibiotic could maintain normal low serum cholesterol level, SGPT, SGOT compared to broiler on antibiotic containing diet (Indriani and Murwani, 2005; Shofianingtyas and Murwani, 2005). The following research was carried out to study the inclusion of MOS, inactivated baker yeast, or combined inactivated...
Table 1: Composition and nutrient contents of experimental diets

<table>
<thead>
<tr>
<th>Feed ingredients (%)</th>
<th>D_x (feed label)</th>
<th>D_y</th>
<th>D_z</th>
<th>D_yz</th>
<th>D_yz+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>NA</td>
<td>32.45</td>
<td>32.45</td>
<td>32.65</td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>NA</td>
<td>3.48</td>
<td>3.28</td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td>Mungbean</td>
<td>NA</td>
<td>39.50</td>
<td>39.50</td>
<td>39.50</td>
<td></td>
</tr>
<tr>
<td>Rice meal</td>
<td>NA</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>Fish protein</td>
<td>NA</td>
<td>11.20</td>
<td>11.00</td>
<td>11.00</td>
<td></td>
</tr>
<tr>
<td>MOS</td>
<td>NA</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Baker yeast</td>
<td>NA</td>
<td>-</td>
<td>0.50</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Noni leaf extracts</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>Water extract</td>
<td></td>
</tr>
<tr>
<td>Vitamins, Minerals and Ailzyme</td>
<td>NA</td>
<td>7.70</td>
<td>7.70</td>
<td>7.70</td>
<td></td>
</tr>
<tr>
<td>Lysine, Methionine and Choline</td>
<td>NA</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

**Nutritive values**

| ME (kcal/kg) | 3071 | 2939.00 | 2936.00 | 2937.00 |
| Crude protein (%) | 22.7 | 21.40 | 21.50 | 21.40 |
| Crude lipid (%) | 3.7 | 1.00 | 1.66 | 1.00 |
| Crude fiber (%) | 2.8 | 2.30 | 2.40 | 2.30 |
| Total Methionine (%) | NA | 0.50 | 0.50 | 0.50 |
| Total Lysine (%) | NA | 1.10 | 1.10 | 1.10 |
| Total Choline (%) | NA | 0.14 | 0.14 | 0.14 |
| Total Ca (%) | 1.4 | 1.30 | 1.20 | 1.00 |
| Total P (%) | 0.9 | 0.70 | 0.70 | 0.70 |

* D_x was composed of Corn, soybean meal, MBM, CGM, Palm Olein, Essential Amino Acids, Essential Minerals, Premix and Vitamins. NA: the percentage of each ingredients in the diet was Not Available. Lipid and Crude fibre of commercial diet were analyzed by proximate analyses.

Vitamin contents per kg vitamin mix: 6000000 IU vitamin A, 1200000 IU vitamin D3, 2.5 IU vitamin E, 3 g vitamin K, 2 g vitamin B1, 3 g vitamin B2, 1 g vitamin B6, 2 mg vitamin B12, 20 g vitamin C, 15 g Niacin, acid, 5 g Ca-D Pantothenate, 750 g Na, Ca, K and Mg electrolyte. This mix was calculated and used accordingly to provide more than adequate level of vitamins (NRC, 1994).

* Based on calculated ME.

* Calculated based on local feed composition table (Harjadi et al., 1986) or proximate analysis.

* Calculated based on feed composition table and known supplemented lysine, methione and choline.

* Total calcium of all diets were analyzed by AAS and total P by Spectrophotometer (AOAC, 1984).

baker yeast and Noni leaves extracts in corn-mungbean base diet on broilers blood biochemical indices and productivity. It is hoped that the results of this study provide more information on the benefit of such additives in broilers diet.

**MATERIALS AND METHODS**

**Diets and broilers:** Local corn, sorghum and mungbean with approximately 11-12% moisture content were ground separately and stored in plastic drum until mix. Local fish egg meal with 56% protein was used as animal protein. Fresh Noni (Morinda citrifolia) leaves were extracted with drinking water in a blender, filtered and mixed directly with the basal diet. Heat inactivated baker yeast was prepared and then directly mixed with basal diet. The basal diet was based on corn and mungbean. Other feed ingredients i.e. sorghum, fish egg meal, vitamin and mineral mix (Medion Indonesia), Ailzyme (Altech, Indonesia), methionine, lysine and choline were used to complete the basal diet and to provide nutrient requirement of broilers as recommended by SNI (2006) (Table 1).

Treatment diets consisted of D_x (commercial/control diet); D_y (basal diet + BioMOS (Altech, Indonesia)); D_z (basal diet + inactivated baker yeast S. cerevisiae (Fermipan)); D_yz (basal diet + inactivated yeast and Noni leaves extracts). The basal diet and each additive were mixed, pelleted, dried and crumbled and each treatment diet was kept in separated and labeled clean plastic drums for feeding. The protein level of the diets were approximately 21% with calculated ME of 2900 kcal/kg. A total of 180 Lohman MB-202 one day old unsexed broilers with average initial uniform body weight of 43-44 g were allocated randomly into the 4 treatment diets (Table 1). The chicks were kept in a warm brooder and given ad libitum access to the diet and drinking water during 21 days experiment. Birds were vaccinated by combined ND La Sota and AI vaccine (PT. Medion Indonesia) on day-4.

**Feed intake, feed conversion and body weight:** Feed intake was recorded daily by subtracting the amount of feed given each day (ad libitum) to each replicate birds with the amount remained. Feed conversion ratio was calculated from feed intake divided by final body weight. Birds on each replicates were weight weekly on 5 kg scale.

**Blood sample collection:** On day 21, one bird of each replicates was sampled randomly for blood collection. Blood was divided into two tubes. First tube was prepared with EDTA and the blood was used for
The determination of hemoglobin and hematocrit. The second tube was stood for 1 h, spun in centrifuge and the resulting serum was collected and kept frozen until analyses. Serum was used for determination of serum GPT, GOT, creatinine, alkaline phosphatase, triglyceride, cholesterol, HDLchol and lactic acid.

**Hemoglobin:** Hemoglobin concentration was determined by Cyanmet-hemoglobin method. Twenty microlitres of blood was mixed with 5 ml Drapkin solution. The mixture was read in spectrophotometer at wavelength 546 nm. Drapkin solution was used as a blank with correction factor of 36.8 (Indonesian Ministry of Health, 1992).

**Hematocrit:** Hematocrit value was determined by Microhaematocrit method. Micro-capillary tubes were filled with blood until ¼ volume and the mouth was sealed. The tubes were centrifuged at 16000 rpm for 3-5 minutes and the resulting blood separated into three layers i.e. red blood cell, buffy coat and plasma. The height of red blood cell was measured by Janetzki scale (Indonesian Ministry of Health, 1992).

**SGPT, SGOT and creatinine analysis:** The activity of Serum Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and creatinine were measured by Kinetic method (Human Gesellschaft, Germany).

**Lactic acid:** Lactic acid was measured by lactic acid Test Kit (Electroquant, Germany). In principle lactic acid is oxidized by NAD under the cataytic effect of lactic dehydrogenase to a pyruvate. The resulting NADH reduced the tetrazolium salt to blue formazan which could be determined reflectometrically. Serum was diluted as needed and the lactic acid test strip was dipped into the diluted serum. The strip was drained of excess serum and inserted into the reflectometer and read. The reading value must fall within the standard range of 3-60 mg/L lactic acid which had been set before hand.

**Serum alkaline phosphatase:** Serum alkaline phosphatase was determined by p-nitrophenol method. Colourless paranitrophenyl phosphate was hydrolyzed by alkaline phosphatase at pH 10.5 and 37°C to form free yellow paranitrophenol. The addition of NaOH stopped the enzyme activity and the absorbance of the resulting colour was measured at 410 nm (WHO, 2006).

**Blood lipids:** Triglyceride was determined by "glycerol-3-phosphate-oxidase para-aminophenazone" (GPO - PAP) "enzimatic colorimetric" method (DiaSys, Germany). Serum cholesterol was determined by cholesterol-oxidase para-aminophenazone (CHOD-PAP) enzymatic colorimetric method (DiaSys, Germany). HDLchol was determined by enzymatic colorimetric after precipitation of β-lipoprotein with phosphotungstate-MgCl₂ acid method (DiaSys, Germany). VLDL+LDLchol was calculated by subtracting total cholesterol with HDLchol (Santoso et al., 2005).

**Relative internal organ weights and abdominal fat:** On day 21, one bird of each replicates was sampled randomly and sacrificed. Abdominal fat and internal organ i.e. liver, heart and kidney were collected and weighed. Abdominal fat was calculated from abdominal fat weight divided by body weight and multiplied by 100%. Relative organ weight was calculated from organ weight divided by body weight and multiplied by 100%.

**Statistical analysis:** A completely randomized design with 4 treatments and 5 replicates was employed. Each replicate consisted of 9 birds. All data were analyzed by ANOVA and Duncan’s multiple range test was used when means were significantly different (p<0.05).

**RESULTS**

Feed intake of broilers fed corn-mungbean base diets were lower than control diet (p<0.05). However feed intake in D₆ and D₇-L were lower than D₇ (p<0.05). Body weight and feed conversion of broilers fed corn-mungbean base diets were lower than control diet. Control diet had the highest body weight and the lowest feed conversion among the treatments.

A significant effect was found for serum GPT, creatinine, triglyceride, cholesterol, VLDL+LDLchol and HDLchol. Serum GPT in D₇-L was lower than D₆ and D₇ (p<0.05), however they were same as control diet (p>0.05). Serum creatinin in D₇ was lower than D₇-L and control diet (p<0.05). D₇-L and control diet had the lowest serum triglyceride. Serum cholesterol value in D₇-L was the lowest, but it was not different than D₇. The highest serum cholesterol was found in D₆ and it was the same as D₇ and D₆ (p<0.05). Serum VLDL+LDL in D₇ and D₆ were lower than D₆ and D₇-L (p<0.05). On the contrary, serum HDL in D₆ was higher than D₇-L (p<0.05). No effect was found for other biochemical indices.

For relative internal organs weight, a significant effect was found for heart, liver, ileum, duodenum and pancreas (p<0.05) (Table 2). In general the relative organ weight for heart, liver, ileum, duodenum and pancreas of broilers fed corn-mungbean base diet were higher than control diet.

**DISCUSSION**

Broiler is a meat type chicken which can produce meat in a short period of time. Modern broiler today reached market weight in a shorter period than before. The rapid growth must be supported by a good quality of diet. Feed intake in this study which used natural additive was not
Table 2: Biochemical indices and productivity of broilers at 21 days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D&lt;sub&gt;c&lt;/sub&gt;</th>
<th>D&lt;sub&gt;h&lt;/sub&gt;</th>
<th>D&lt;sub&gt;l&lt;/sub&gt;</th>
<th>D&lt;sub&gt;vL&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g)</td>
<td>1175.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1004.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1069.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1023.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>896.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>605.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>624.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>617.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>1.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.86&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>8.26</td>
<td>8.31</td>
<td>7.95</td>
<td>8.53</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>28.60</td>
<td>28.80</td>
<td>29.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Lactic acid (ppm)</td>
<td>824.40</td>
<td>529.00</td>
<td>641.00</td>
<td>456.00</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>11.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>140.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.84</td>
<td>170.92</td>
<td>151.59</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (U/L)</td>
<td>59.08</td>
<td>108.00</td>
<td>99.20</td>
<td>112.20</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>38.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>135.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum VLDL+LDL (mg/dl)</td>
<td>23.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>113.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abdominal fat (%)</td>
<td>1.01</td>
<td>1.27</td>
<td>1.21</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Relative organ weight (%)

- Heart: 0.55<sup>a</sup> 0.83<sup>a</sup> 0.74<sup>a</sup> 0.79<sup>a</sup>
- Liver: 2.55 3.12<sup>a</sup> 3.88<sup>a</sup> 4.50<sup>a</sup>
- Kidney: 2.32 1.91 1.85 1.81
- Gizzard: 1.96 2.29 2.22 2.18
- Ileum: 1.54<sup>a</sup> 2.27<sup>a</sup> 2.39<sup>a</sup> 1.82<sup>a</sup>
- Jejunum: 2.15 2.51 3.00 2.61
- Duodenum: 0.73<sup>a</sup> 1.00<sup>d</sup> 1.04<sup>a</sup> 1.16<sup>a</sup>
- Pancreas: 0.26<sup>a</sup> 0.42<sup>a</sup> 0.39<sup>a</sup> 0.41<sup>a</sup>

significantly different among broilers fed basal diets. However feed intake of broilers fed basal diet was significantly lower than control diet. Lower feed intake is most probably due to the presence of vegetable feed ingredients as the major components of the diet. These feed ingredients contained anti nutritive components that were difficult to digest by endogenous enzymes which included Non-Starch Polysaccharides (NSP) and phytates (Chitra et al., 1998; Mubarak, 2005). In feed enzyme was used to break the polymeric chain of NSP into simpler ones to improve the nutritive value of feed ingredients. However, the use of recommended dosage of feed enzymes into corn-mungbean base diet did not affect broilers performance. This was reflected in final body weight and feed conversion which were lower than control broilers. Our results were in line with another study by Iji et al. (2001), which used lupines as vegetable protein in broiler diet. It showed similar results on body weight, feed intake and feed conversion at 21 days which were 516.2-580.9 g, 1150-1345 g and 2.19-2.31 respectively.

Further, the standard of metabolizable energy for broilers according to the feed manufacture brochure (control diet) is approximately 3000-3100 kcal/kg. In vivo ME determination of control diet at older age (35 days) showed a higher value (± 3300 kcal/kg) than calculated value. In our study we made calculated ME of basal diet according to SNI (2008) i.e. 2900 kcal/kg which was lower than control diet (Table 1). Lower ME of corn-mungbean base diet was expected would increase feed intake. However, this was not the case. The control broilers which had higher ME had higher intake. Therefore the general knowledge that birds consume feed to meet their energy requirement is not applicable to modern broiler. Modern broilers today are the results of selection which was originally selected on the basis of high feed intake. It appeared in our case that higher ME also produced higher intake (D<sub>c</sub>). This case was also found by others and it was suggested that feed intake depended not only on ME value but also to the first limiting nutrient (Mbaijorgu et al., 2011). In our case and in Iji et al. (2001) as previously mentioned, the limiting nutrient would be NSP which limited the digestion of carbohydrates in the diet and led to low dry matter digestibility and reduced feed intake. Dry matter digestibility measurement of broiler diet at 35 days old showed lower value than control diet at the same age. Consequently, the body weight and feed conversion also declined. A follow up experiment to address this issue is undergoing.

On the other hand, the rapid growth of broiler also brings some consequences (Hunton, 2006; Murwani, 2010). One consequence is requirement for good supply of oxygen to support rapid synthesis of muscle protein. Other consequences are the occurrence of high abdominal fat, incidence of feet disorders such as Tibial Dyschondroplasia (TD) and ascites. One of the causes in the incidence of ascites is due to the high demand of oxygen supply. The size of broilers heart is small compared to its body size and this imbalance could compromise blood circulations. Therefore we thought that the heavy workload of supplying oxygen for the rapid synthesis of muscle protein can be supported via antioxidants containing feed ingredients such as used
in our study. We also speculated that such feed ingredients could also help the growth of digestive and other organs during the fastest growing period (starter). From our results it could be seen that different additives did not affect the levels of Hemoglobin, Haematocrit that were already in the normal range and was not different than the control diet. A complete diet with sufficient protein level and trace elements required for normal synthesis of Hemoglobin was provided so that Hemoglobin and Haematocrit was normal. For serum lactic acid, although there was no significant difference among treatments, it was noteworthy that broilers receiving diet plus combined yeast and Noni leave extract (D<sub>1-5</sub>) had the lowest value. During extraction of energy from glucose in the glycolytic pathway, lactic acid was formed when oxygen supply was low or less available in the tissue. We speculated that tissue oxygenation in corn-mungbean fed broilers was better resulting in lower serum lactic acid. As a note, the temperature inside broiler house was 27°C at dawn; 30°C at 06:00 to 09:00 a.m, 34-37°C at 10:00 to 14:00; 31°C in the afternoon and 29°C at night. The temperature throughout the day varied widely and during pronounced stress, oxygen demand would be higher.

Interestingly SGPT value of broilers in D<sub>1-5</sub> was also low and similar to broiler in control diet. SGPT is a general indicator of the workload of the liver. When SGPT was viewed by its relationship with relative liver weight, it could be seen that it was also the greatest in D<sub>1-5</sub>. Even though greater value of relative liver weight could also be due to smaller numerical value of body weight as the dividing factor, we speculated that greater relative weight might be associated with the development of liver as accessory organs during starter period. Furthermore, gross anatomical observations of the livers were normal in all broilers. Therefore it might be thought that feed ingredients in corn-mungbean base diet might play role in liver development and this development is enhanced by the presence of Noni leave extract in D<sub>1-5</sub> broilers. Further study is needed to test this idea. When linked to relative heart weight, corn-mungbean fed broilers also showed a greater value than control diet. This also led us to think that the types of feed ingredients can affect heart development. Although feed ingredients are needed to support rapid growth by providing required nutrients, it should be in balance with the capability of supporting organs (size and weight). Otherwise, it will be detrimental to the body. As the size of broiler heart is small compared to its large body size it might be beneficial to use certain feed ingredients to reduce this weakness.

Serum GCT and AFA could be used as indicators of pathological events in the heart. As all values were not different, it indicated a normal condition in all treatments. Gizzard as an organ where grinding of feed materials taking place showed no significant difference in their relative weight. It implied that the texture of all diets were the same and therefore it did not affect gizzard development. The relative weight of small intestines showed a greater weight for ileum and duodenum in corn-mungbean base diet compared to control diet. Pancreas as producer of pancreatic enzymes also had a significantly higher relative weight in corn-mungbean base diet than control diet. Greater relative weight of these organs was thought to be the same as for liver and heart as discussed previously. The weight of the kidney as an organ of excretion was not affected by the diets and it indicated that secretion of metabolic products via kidney was normal. This was also shown in the levels of creatinine which was similarly low although the lowest one was found in D<sub>1</sub>.

It is interesting to note that although feed intake in D<sub>1</sub>, D<sub>2</sub> and D<sub>1-5</sub> are the same, their lipid profiles have a significant difference. It was found that triglyceride levels was lowest in D<sub>1-5</sub>. Serum triglyceride is the source of lipid deposits and adipose tissue development including abdominal fat depends on the availability of serum triglycerides (Hermier, 1997). Consequently, when circulating serum triglycerides was low, the abdominal fat would be low. The abdominal fat in this study which ranged 1.01-1.27% was much lower compared to that found by Murvani and Baywardhi (2007) in which abdominal fat reached 3%. It was interesting to note that triglyceride level and percentage of abdominal fat in control broilers (D<sub>0</sub>) was similarly low to D<sub>1-5</sub>. As a note this study did not use antibiotic via drinking water. Therefore there was no influence of antibiotic in lipid metabolism. Low level of triglyceride in D<sub>1-5</sub> could be due to the role phytobiotic Noni leaf extract which could reduce serum lipid (triglycerides, total cholesterol and LDLChol). The mechanism by which the leaf extract reduced serum triglyceride was thought to be mediated by inhibition of biosynthesis and secretion of lipid by the antioxidative phytochemicals in Noni leaf extracts (Mian-Ying et al., 2002; Mandukhail et al., 2010). On the other hand, similar level of serum triglyceride in the control broilers was thought to be due to the presence of palm olein in the diet. The content of total fatty acids in palm olein are approximately 40% palmitic acid, 43% oleic acid (monounsaturates/MUFA) and 10.5% linoleic acid (polyunsaturates/PUFA) (Choudhury et al., 1995). MUFA and PUFA have been well studied to affect lipid metabolism and reduce serum lipid (Berry et al., 1991). As broilers have characteristic of high feed intake, it was expected that in spite of high feed intake, the adipose tissue could remain low when circulating triglyceride was low. This was shown by low level of serum triglyceride level and percentage of abdominal fat in control broilers (D<sub>0</sub>) which was similar to D<sub>1-5</sub>.

The treatment diets also produced a significant difference in serum total cholesterol. Serum total
cholesterol of broilers receiving the combined inactivated baker yeast and Noni leaf extract (DLN) was lowest among the treatments. As the difference of DLN to other treatments was due to the presence of Noni leaf extract, it appeared that the phytochemicals in Noni leaf extract could play role in lowering total serum cholesterol (Mian-Ying et al., 2002; Mandukhail et al., 2010). Noni leaf extract could exert similar mechanism in lowering serum cholesterol as triglycerides as previously discussed. However, another factor could also play role in the regulation of cholesterol lowering namely uptake by peripheral tissues and clearance by the liver. It has been known that uptake of cholesterol by peripheral tissues from LDLchol was mediated by high affinity of LDL receptor (LDLr) (Hayashi et al., 1989). The expression of LDLr was found to correlate negatively with serum LDL and percentage of abdominal fat. When LDLr was higher, serum LDL and abdominal fat percentage was lower (Musa et al., 2007). Serum cholesterol uptake by peripheral tissues and clearance by liver could be different between DL and DLN. Lower serum total cholesterol in DLN could be due to enhanced clearance of HDLchol by liver. It has been shown that dietary plant derived polyunsaturated fatty acids stimulated hepatic HDLr and HDL cholesterol ester uptake (Kozarsky et al., 1997; Spady et al., 1999; Trigatti et al., 2003). Noni leaf was known to contain a large number of functional phytochemicals which could play role in LDLr mediated lowering of serum total cholesterol in DLN and shown by lowest serum HDLchol. Such low HDLchol was also found in rat studies by Dorfman et al. (2005) and Mandukhail et al. (2010). Other findings by Alzaqwar et al. (2010) and Rehman et al. (2011) also showed low level of HDLchol in broilers i.e. 54.69.67 mg/dl and 33.33–72.16 mg/dl respectively. Furthermore, the ratio of total cholesterol to HDLchol in this study was also lowest in DLN, supporting the role of Noni leaf extract in modulating serum HDLchol or lipid metabolism in broilers. Such low HDLchol in broiler is interesting and not necessarily disadvantageous as cholesterol homeostasis was regulated by various biochemical pathways. However, a careful consideration must be exercised.

On the other hand, lower serum total cholesterol in DLN could be due to enhanced uptake of HDLchol by peripheral tissues via LDLr. This was shown by lowest serum VLDL+LDLchol in DLN. It appeared that inactivated baker yeast which could provide different oligosaccharides and other active micro-chemicals other than MOS, may increase LDLr in peripheral tissues and hence lowering VLDL+LDLchol in our study (Fukushima et al., 2000). Other study in broiler which used medicinal plant blends in diets also showed low VLDL+LDLchol i.e. 32.48–69.72 mg/dl (Khaligh et al., 2011). Another study in rat which used various parts of Noni also showed low level of VLDL+LDLchol i.e. 26.7, 27.33, 41.04 mg/dl for fruit, root and leaves extract respectively (Mandukhail et al., 2010). However, very low VLDL+LDLchol in broilers fed control diet could only be speculated to be due to PUFA containing vegetable oil used in the control diet.

Our findings here indicated that modern broilers with the tendency of having high serum lipid and abdominal fat can be prevented by nutritional intervention i.e. supplementation of natural additives. Our results provide evidence that MOS, or inactivated baker yeast alone, or in combination with Noni leaf extract in com-mungbean base diet produce low serum lipid and abdominal fat in broilers with no negative effect on other serum biochemical indices. Further studies to improve productivity is underway.

ACKNOWLEDGEMENT

This study was supported by Program Hibah Kompetensi, Directorate General of Higher Education Indonesia year 2008-2010 which was granted to Retro Murwani as the single Principal Investigator. Thank you to Alltech Indonesia for Allzyme and MOS for this study.

REFERENCES


