The Effect of Used Form and Level Green Cincau Leaves (Cycleabarbata L. Miers) as Feed Additive on Broiler Performance Production

Muhammad Halim Natsir¹, Osfar Sjofjan¹ and Muharlien²
¹Department of Animal Nutrition, Faculty of Animal Husbandry, University of Brawijaya.
²Department of Animal Production, Faculty of Animal Husbandry, University of Brawijaya.
Email: emhanatsir@ub.ac.id

ABSTRACT
The aimed research was to examine the effect of used form and level green cincau leaves (Cycleabarbata L. Miers) as feed additive on broiler performance (feed consumption, body weight gain, feed conversion ratio and income over feed cost). The materials used were 192 Day Old Chicks (DOC) of Lohman MB Platinum with average initial body weight of 37.4±2.87 g, encapsulated or nonencapsulated green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%. Encapsulants that used was 25% of green cincau leaves (Cycleabarbata L. Miers) and encapsulants. Encapsulants that used was the mixture of gum arab-whey (4:1) which was added BHT 0.06%.

The conclusion of this research was encapsulated better than nonencapsulated green cincau leaves (Cycleabarbata L. Miers). The optimal level of feed additive in encapsulated green cincau leaves (Cycleabarbata L. Miers) was 1.0%.

Keywords: green cincau leaves, Cycleabarbata L. miers, encapsulated, broiler performance.

INTRODUCTION
The use of antibiotics as feed additives has been widely used as livestock growth boosters be optimized and fast, especially widely used in commercial feed. Bahari et al. (2012) most of the animal feed produced by feed factory content livestock medicated especially the antibiotic growth promoters (AGP). The use of antibiotics as feed additives are very effective for improve efficiency of feed by pressing down the growth of microbial pathogen in the intestine and improve the appearance of the intestinal villi so it can be maximize the digestibility of feedstuff substance of feed consumption to fulfillifes needed and production of broiler, but the results of these antibiotics can leave residues in carcass that unsafe to human consumed, so it is necessary to use natural ingredient contain phytobiotics to make feed additive.
One of the plants that can be used as the phytobiotic is green cincau leaves. Heyne (1987) green cincau leaves contain chemical compounds such as alkaloids, flavonoids, chlorophyll, carotenoid and saponins. Green cincau leaves can be used as a feed additive in powder or nonencapsulated and encapsulations of green cincau leaves extract. The form of encapsulated and nonencapsulated allegedly can produce different effects against the appearance of broiler production.

**MATERIALS AND METHODS**

One hundred and ninety-two unsex day old Lohman broiler chicks from local Hatchery were used with uniform initial body weight of 37.4 ± 2.87 g and coefficient of diversity 7.67%. They were randomly allotted to 24 experimental units. Each experimental flock unit was of 100x100x70 cm in sizes and used for 8 chicks till 35 days of age, equipped with waterer and feeder and raised on litter floor.

Encapsulations material was used gum Arab-whey with a 4:1 comparasion of as much 25% weight of green cincau leaves extract. 0.06% Butylated Hydroxy Toluene (BHT) was added in encapsulation process. The analyzed composition of basal diets used show in Table 1.

The experiment was designed based on Nested Completely Randomize Design (CRD) with 2 main factors, namely form and level of phytobiotic. The forms comprised of powder or nonencapsulated and encapsulated forms, while levels of phytobiotic consisted of 0% (L0), 0.5% (L1), 1.0% (L2) and 1.5% (L3) of phytobiotic added to the basal diet. Each treatment was repeated 3 times with 8 chicks each. Feed and water were given ad libitum till 35 days of age.

The performance measured included feed consumption (FC), Body Weight Gain (BWG), Feed Conversion Rate (FCR), Income Over Feed Cost (IOFC) and Production Index (PI).

Data were then analized by Nested Completely Randomize Design (RAL) analysis variation (ANOVA) and if there was significant effect followed by Duncan’s Multiple Range Test (Steel and Torrie, 1993).

**Table 1. The analyzed composition of basal diets.**

<table>
<thead>
<tr>
<th>Nutrients composition*</th>
<th>Starter</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter (%)</td>
<td>89.92</td>
<td>91.03</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>23.16</td>
<td>20.84</td>
</tr>
<tr>
<td>Crude Fiber (%)</td>
<td>3.10</td>
<td>3.88</td>
</tr>
<tr>
<td>Crude Fat (%)</td>
<td>6.57</td>
<td>7.28</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.92</td>
<td>6.86</td>
</tr>
<tr>
<td>ME (kcal/kg)**</td>
<td>3061.39</td>
<td>3127.63</td>
</tr>
</tbody>
</table>

* Analysis result in Animal Nutrition Laboratory, Animal Husbandry Faculty, University of Brawijaya.

** Laboratory calculation analysis result of ME based on 70% GE (Patrick Schaible and, 1980).

**Experimental Model of Metabolic Syndrome.**

After 2 weeks of dietary manipulation, rats were injected intraperitoneally with STZ (30mg/kg). The body weight and biochemical parameters (blood glucose, triglyceride, HDL-cholesterol) were measured 7 days after the vehicle or STZ injection; the HFHS diet was continued for the rest of 6 weeks of dietary manipulation in rats. The rats with blood glucose (>126mg/dL), triglyceride (>150mg/dL), high systolic blood pressure (≥140 mmHg), and reduced HDL levels (<40 mg/dL) confirmed presence NCEP-ATP III criteria of metabolic syndrome (Grundy, 2004). Thereafter the rats were either fed normal diet or HFHS diet as per the protocol for 8 weeks. Blood samples were collected from the tail veins under light
anesthesia at 2, 4, 6, and 8 weeks for estimation of biochemical parameters.

**Experimental Groups.**

Group 1: Normal Control (NC). In Normal Control group, rats were administered distilled water orally using a feeding bottle during study period of 8 weeks. At the end of 2 weeks, 0.01M citrate buffer, pH 4.5, was injected intraperitoneally to mimic the STZ injections.

Group 2: High Fat and High Sucrose Diet (HFHS). The high fat diet and high sucrose diet were fed to rats for 8 weeks to produce metabolic syndrome. At the end of second week rats was induced by a single STZ injection (30 mg/kg body wt, i.p., dissolved in 0.01M citrate buffer, pH 4.5).

**Physiological Measurement.**

Daily food intake, fluid intake, and calorie intake were measured every day and Body weight was measured every week for eight weeks. The food and fluid intake for each rat were measured by subtracting the measured amount provided to the remaining amounts in the cage [11].

**Blood Biochemistry.**

The rat blood samples of all experimental groups were collected from the tail veins under light anesthesia at 2, 4, 6, and 8 weeks for estimation of fasting blood glucose, Triglyceride, and HDL level. In addition, after the completion of the experimental duration (8 weeks), serum was used for the determination of insulin level.

**Blood Pressure Measurements.**

Blood pressure was measured using the tail-cuff method with sphygmomanometer technique at the baseline and at the end of the experiment. Three readings were taken consecutively and the average was then calculated and taken as a final reading for SBP.

**Statistical Analysis.**

All data was analyzed with Statistical Package for Social Sciences (SPSS, version 22) and were presented as mean values with their standard error of means (SEM) and subjected to Independent T-Test and pair T-Test with significant $P$ value as <0.05.

**RESULTS**

**Physiological Parameter.**

Table 1 showed the effect on physiological parameters following consumption control diet and High fat diet and high sucrose diet with low STZ. The food intake and fluid intake were increased significantly with the consumption high fat diet and high sucrose diet with low STZ after eight weeks and there was statistical difference compared to control group. On the other hand, the body weight was significantly increased in the intervention group after eight weeks period followed by control group and no significantly difference between groups.

**Blood Pressure.**

Intervention groups showed significant blood pressure increase after eight weeks compared to that of the baseline. The final systolic blood pressure also revealed a significant higher level of systolic blood pressure compared to that of control group (Table 2).

**Blood Biochemistry.**

Fasting blood glucose and TG levels of intervention group showed significant increase during eight weeks and differed significantly compared to that of control group (Table 2). HDL levels of intervention group showed significant decrease during
eight weeks and differed significantly compared to that of control group (Table 2). On the other hand, no significant difference was observed in the fasting glucose, TG and HDL level among control group. Insulin level and HOMA IR index showed significantly different between these groups.

Table 2. Effect of high fat and high sucrose diet with low dose STZ on body weight, food intake, fluid intake in experimental group for eight weeks

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal Control</th>
<th>HFHS group (High fat and High sucrose with low dose STZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 weeks</td>
</tr>
<tr>
<td>Body weight</td>
<td>289.67±16.02</td>
<td>400.22±25.11*#</td>
</tr>
<tr>
<td>Food intake</td>
<td>21.48±0.43</td>
<td>22.06±0.62</td>
</tr>
<tr>
<td>Fluid intake</td>
<td>29.74±5.06</td>
<td>22.06±0.62</td>
</tr>
</tbody>
</table>

*: significant between baseline and after 8 weeks (p<0.05).
#: significant between group (p<0.05).

Table 3. Effect of high fat and high sucrose diet with low dose STZ on metabolic variable in experimental group for eight weeks

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal Control</th>
<th>HFHS group (High fat and High sucrose with low dose STZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 8 weeks</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>92.22±14.86</td>
<td>86.56±16.05</td>
</tr>
<tr>
<td>Triglyceride level</td>
<td>105.56±35.15</td>
<td>115.22±28.38</td>
</tr>
<tr>
<td>HDL level</td>
<td>49.78±7.67</td>
<td>44.33±2.18</td>
</tr>
<tr>
<td>Insulin</td>
<td>-</td>
<td>2.52±0.38</td>
</tr>
<tr>
<td>Homa-IR Index</td>
<td>-</td>
<td>1.46±0.40</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-</td>
<td>125.57±25.14</td>
</tr>
<tr>
<td>SBP (systolic Blood Pressure)</td>
<td>124±4.2</td>
<td>126.89±5.4</td>
</tr>
</tbody>
</table>

*: significant between baseline and after 8 weeks (p<0.05).
#: significant between group (p<0.05).
Figure 1. Time course changes of blood glucose level of NC ($n = 9$), HFHS group ($n = 9$). Values are expressed as mean ± SD, *: significant between baseline and after 8 weeks ($p<0.05$), #: significant between group ($p<0.05$), NC versus HFHS.

Figure 2. Time course changes of Triglyceride level of NC ($n = 9$), HFHS group ($n = 9$). Values are expressed as mean ± SD, *: significant between baseline and after 8 weeks ($p<0.05$), #: significant between group ($p<0.05$), NC versus HFHS.
DISCUSSION

This study developed metabolic syndrome based on NCE-ATP III criteria that characterized by hyperglycemia, insulin resistance, elevated blood pressure, and dyslipidemia. There are several animal models of diabetes as well as metabolic syndrome. Some of them were genetically modified such leptin-deficient (ob/ob) mice, leptin receptor deficient (db/db) mice, Zucker fatty (ZF) rats, Zucker diabetic fatty (ZDF) rats, Dahl.S-Z-Leprfa/Leprfa (DS/obese) rats, Goto-Kakizaki (GK) rats, obese spontaneous hypertensive rat (Koletsky rat), and the POUND mice (Wong, Chin, Suhaimi, Fairus, & Ima-Nirwana, 2016). Moreover, there were some diet induced metabolic syndrome rat model such as high-fructose, high-sucrose, high-fat, high-fructose/ high-fat, or high-sucrose/high-fat diets (Gajda, Pellizzon, Ricci, & Ulman, 2007). Furthermore, some chemicals might induce metabolic syndrome such as alloxan, streptozotocin, glucocorticoid agent (Bertram & Hanson, 2001). Further, it was kept in mind while developing the model that it should be less expensive, easily available, taking relatively short periods for development, reproducible, and displaying the various components of metabolic syndrome and diabetes mellitus. This study comply those criteria and suggested a novel approach in inducing rat metabolic syndrome characteristics through combination of low dose streptozotocin and high fat diet derived from hydrogenated fat.

Physiological Parameters such as body weight, food intake, fluid intake were evaluated in the healthy normal control (NC) and High Fat and High Sucrose (HFHS) groups. At the end of study, the NC group body weight was higher than that of HFHS group. Similarly, the Food intake and Fluid Intake of the HFHS
group also increased significantly as compared to the NC rats at similar time points. This result was similar with (Novelli et al., 2007) and (Suman, Ray Mohanty, Borde, Maheshwari, & Deshmukh, 2016) that showed an increase in all physiology parameters until the end of the study. Moreover they showed that diabetic state induced by insulin resistance caused weight loss, increase food intake and fluid intake increase.

**Development of Hyperglycemia.**

The present study suggested the development of hyperglycemia after streptozotocin and HFHS diet administration. It was characterized by high fasting blood glucose and HOMA-IR index. The blood glucose level in the HFHS group rats was significantly increased as compared to that of the baseline. Moreover, the blood glucose of HFHS group was significantly higher than that of NC group at the end of study. As explained by (Kuate et al., 2015), high carbohydrate and fat diet only would not induce frank hyperglycemia. Moreover, (Wang et al., 2007) revealed that hyperglycemia appeared at 3-4 weeks subsequent to streptozotocin administration. We hypothesized that the metabolic changes were manifested after a longer duration.

Insulin resistance and pancreatic function decline were demonstrated by a significant higher HOMA IR index in HFHS group as compared to NC group rats. Some studies revealed that streptozotocin injection resulted in diabetes type 1 it is due to massive pancreatic injury. However multiple low dose streptozotocin induced mild to moderate pancreatic damage that resulted in diabetes type 2 animal model (Arshad, 2013). However, this study induced diabetes type 2 only without any evidence of dyslipidemia and hypertension. Our study use the combination the streptozotocin and high fat diet (40%) fat to maintain the hyperglycemic state. Previous report suggested that HFD administration induce insulin resistance via PKC activation by high level free fatty acid that consequently interfered IRS activation (Jang, 2013). Moreover, our study used the combination of animal fat and hydrogenated fat to maintain insulin resistance. Previous study explained that trans fatty acid ingestion induced insulin resistance ingestions could be related to the innate immune cascade in adipose tissue that promote pro-inflammatory adipokines expression increase (Ibrahim et al., 2005). Furthermore, NF-kB, a key transcription factor in inflammatory cascade coordinated pro-inflammatory adipokines in adipose tissue (Aldhahi, 2003). Previous study also showed TRAF-6 increase that contributes to inflammatory response leading to NF-kB pathway activation (Ibrahim, 2005). Moreover, (Creely et al., 2007) revealed a positive correlation between insulin serum concentration and endotoxinemia. Therefore, it is possible that the hyperinsulinemia presented in TFA group associated with the effect of fatty acids in the intestinal microbial composition (Cani et al., 2008). It modified the intestinal permeability elevating lipopolysaccharides the inflammatory cascade activation. Finally, it promoted increasing TRAF-6. Thus, the combination of low dose STZ and high fat diet could induce diabetes type 2 via insulin resistance.

**Development of dyslipidemia.**

Dyslipidemia is a hallmark of metabolic syndrome. Dyslipidemia was appeared after 8th week induction with HFHS. Abnormal lipid profile in metabolic syndrome was characterized by high triglyceride (>150
mg/dL), and low HDL levels (<40 mg/dL). This study showed significant rises triglyceride level and decrease HDL level compared to that of the baseline in HFHS group. At the end of study, a significant higher triglyceride level and lower HDL level was observed in the HFHS group as compared to that of NC group. Some previous studies also showed the efficacy of high fat and high carbohydrate on raising the triglyceride level and reducing HDL level. Dyslipidemia occurred due to free fatty acid increased after high fat diet and high carbohydrate administration. Free fatty acid could induce inflammation and increase triglyceride and cholesterol metabolism. We hypothesized that different fat sources used in our study progressively induced dyslipidemia. Hydrogenated vegetable fat used in our study promoted an increase in serum triacylglycerol and total cholesterol levels. Long term intake of TFAs altered plasma lipoprotein profile that increased the risk of cardiovascular diseases, decreased insulin sensitivity, and higher risk of type 2 diabetes (Ibrahim et al., 2005; Lichtenstein et al., 2001). Based on our knowledge this study was the first study revealed metabolic syndrome features with the use of fat sources that mimic daily consumed fat in human.

**Development of hypertension.**

Present study showed systolic blood pressure elevation after low dose STZ and HFHS diet administration for 8th week. Previous studies also showed increase in systolic blood pressure in metabolic syndrome induced by high carbohydrate and fructose diet (Bantle, 2009). This condition explained by peripheral resistance increase and renin-angiotensin-aldosterone system (RAAS) activation. RAAS activation plays a key role in the development and pathophysiology of hypertension by several mechanisms such as vasoconstriction, cell growth, oxidative stress production and inflammation via angiotensin II production (Beevers, 2001). Moreover, free fatty acid induced hypertrrophy and hyperplasia of adipocyte. This condition led to proinflammatory mediator release such as angiotensin 2 and suppresion of anti inflammatory mediator such as the adiponectin (Aldhahi, 2003). Our study showed low adiponectin level as important biomarker of metabolic syndrome. Finally, we assumed that the devopment of hypertension in our study was induced by high grade inflammation.

**The distinctiveness Animal Model of Metabolic Syndrome.**

The present study has formulated a unique formulation of HFD which can be prepared indigenously in laboratory and is therefore feasible and cost effective. Thus, the present study has attempted to develop a unique rodent model of metabolic syndrome in the setting of diabetes mellitus. Although presently there is no perfect animal model of these comorbidities, the present study for the first time has successfully developed an experimental model with specific attributes (dyslipidemia, hypertension, and diabetes) that makes them useful for studying the mechanisms and potential therapies of metabolic syndrome in the setting of diabetes.

**CONCLUSION**

The combination of low doses of STZ (30mg/kg) and HFHS administration for 8 weeks could induce metabolic syndrome mimicking human criteria of metabolic syndrome based on NCE ATP III criteria such as hyperglycemia, hypertriglyceridemia, low HDL level, and hypertension. Moreover, low adiponectin level as an important biomarker
of metabolic syndrome was observed in HFHS group. The developed model will be helpful in screening of different pharmacological compounds.

**Conflict of Interests.**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**ACKNOWLEDGMENT**

Authors are thankful to KEMENRISTEK DIKTI and University of Brawijaya for providing the funding support to the present investigation.

**REFERENCES**


