Average Daily Gain, AST and Blood Nitrogen Urea (BUN) Responses of Bali Beef on Cocoa Waste Extract Supplement

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***Abstract*—the research aimed to identify blood plasma AST, BUN, Triglyceride and average daily gain (ADG) responses of Bali beef on cocoa pod husk (CPH) extracts supplementations. 15 males of Bali beef in fattening premises were divided according to feed treatments; group A1 with normal feed; A2 CPH meal (CPH-M); A3= CPH crud (CPH-CE); A4= high theobromine CPH (CPH-T); A5= high polyphenol CPH (CPH-P). Blood collection and body weighting held one day before treatment (B1); B2= 24 h; B3= 14 d; B4= 28 d; 42 d later. Result show that there was no significant difference in ADG values within all treatments, although the control and CPH-P had higher ADG at 14-56d of treatment. The blood AST activity were same (P>0.05) in control, CPH-M and CPH-C and significantly higher (P<0.05) than CPH-T and CPH-P Thus preliminary parameters implied that utilizing of CPH as feed for Bali beef didn't indicate any negative effect.**

***Index Terms*—cocoa waste extract, CPH, Bali beef, ADG, BUN, Urea.**

I. Introduction

Indonesia was the third cocoa producer after Ghana and Ivory Coast (*Côte d'Ivoire)*, more than 712,000 tons/year cocoa produced from about 1.774.463 Ha growing areas in the country. Four provinces with widest plantation areas were in Sulawesi Island, Middle Sulawesi, South, West and South East Sulawesi. 55.3% or 980,711 Ha growing areas were exist in these provinces. South Sulawesi was the second producer after Middle Sulawesi province, in 2012, recorded that Province produced about 176,000 tones and coverage about 60% for nation demand at the years (South Sulawesi Bureau of Statistic, 2013). In the intensive cocoa plantation and industries, beside their beans, they left wastes from the estate plantation to the manufacturing industries. In the estate plantation, large amount of cocoa pod and husk (CPH) are piled up after cocoa beans have been extracted, about 3 Kg dry matters of cocoa pod can be produced for each kilogram of beans. Today, there only few of thus existing untapped potential for utilizing alternative feeds for ruminants. In the past, CPH had not been utilized as a feed ingredient for two main reason: 1) it contains a toxic alkaloid, theobromine, which may have accumulative harmful effect, and 2) its high crude fiber containing, up to 340/Kg dray matter or 16,45% of dry matter consisted [1]. Recently, some works reported so far, suggest that in low levels of CPH can be safely included in the feed ingredient, in poultry; pig, cattle; rabbit, sheep and in goat [2]. As feed ingredient, CPH were mostly given in crude or chopped dried and fermented shape which confined the utilization because of the animal palatability and feed storage problems.

Utilizing agricultural waste, consequence the integration term and presenting the animal in plantation estates. In rural live of Indonesia, cattle were close to the farmers identically. Bali beef is one of endogenous cattle species with well locally adaptation under draft environmental condition and have good reproduction profiles, and have dominated the cattle population outside of Java, primarily in Eastern Indonesia, include South Sulawesi [3]. Most of local research reports suggested that fermented crude cocoa pods to use as feed ingredients and seem its positive effect on average body gain and some reproduction performance in ruminants.

Related to the current issues, however, the research observation in physiological aspect as the effect of CPH are poorly found, also related to shapes which CPH gave as feed and the ruminant species. Therefore more work is needed in order to be able to make firm recommendations on incorporation of CPH in ruminant diets, especially on Bali beef. This research aimed to identify blood plasma profile included blood aspartate transamilase (AST) activity, blood urea nitrogen (BUN) and blood plasma triglyceride, and average daily gain (ADG) responses of beef cattle on cocoa waste extract feeding.

II. Materials and Method

The research conducted in July - November 2013, taken place at a commercial fattening farm in local

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Makassar city, South Sulawesi. Chemical analyses were conducted in Animal Physiology Laboratory of Animal Husbandry Science Faculty of Hasanuddin University.

A. CPH Extraction

The CPH meal used in this trial was obtained from local cocoa plantation estate. After the pods were opened and the beans extracted, the pods and husk were cropped into small pieces and sun dried for 3 days, followed by oven drying for an hour at 50^oC. Crude dried CPH milled until sieved in 20 mashes. A 20g sample was then extracted in hexane (1:5) for 4 times for fat extraction followed by drying in oven. Sieve 100 mesh powder from oven was extracted their polyphenol using 80% acetone, this step was repeated 3 times at 80^oC. The solution was evaporated with vacuum evaporation and drying with freeze-dryer [4]. Theobromine extracts were purchased from Rhino-Pharmeutical.

B. Animals and Experimental Design

15 male Bali beef with relatively same in age (2 - 2.6) and body weight (203.2±18.6 kg) were placed in individual cages. Factorial design was used to arrange the animals with different kind of CPH extract feeds (Factor A): A1= control (feed without CPH extract); A2= CPH meal (CPH-M); A3= CPH crud extract (CPH-C); A4= high theobromine CPH extract (CPH-T); and A5= high polyphenol CPH extract (CPH-P). The second factor is treatment durations (Factor B): B1= before treatment; B2= 24 h after; B3= 14 d after; B4= 28 d after; and 42 d after treatment. The cattle were scaled for daily gain (ADG) parameter at 14, 28, 42 and 52 days. Blood collections for blood profile parameters were taken at before treatment, 1st, 14th, 28th, and 42nd day after treatments. Univariate ANOVA was used to identify the significant effects from interaction of the treatments, and contras analysis was used to identify the differences between CPH feeding treatment and the controls.

C. Blood Sample and Plasma Evaluation

Jugular bloods of samples collected and centrifuge 30 minutes after collection for 15 minutes at 3000 rpm. AST and BUN profiles were evaluated by Roche Diagnostic GmbH, Jerman standard procedures with COBAS C111 [5].

III. RESULT AND DISCUSSION

A. Average Daily Gain (ADG)

After 14 days of treatment, animals with CPH-P show the highest ADG (0.59 ± 0.04), followed by CPH-C (0.49 ± 0.1), Control (0.55 ± 0.02), CPH-M (0.49 ± 0.07) and CPH-T (0.56 ± 0.02). At day of 28th, CPH-C had the highest ADG (0.62 ± 0.07) and followed by CPH-P (0.57 ± 0.04). At the day of 42nd, CPH-P effect back to the highest ADG value (0.6 ± 0.05), with CPH-M and CPH-T were seem have similar value (0.58 ± 0.05). The last, after 56 days of treatment, the controls animals appeared the highest ADG value (0.63 ± 0.05), and followed by CPH-P, CPH-T, CPH-C and CPH-M (Fig. 1). Generally, ANOVA analysis showed that there is not significant effect (P>0.05) from the kinds of CPH extracts and their interactions with the treatments period on the ADG. When comparing the ADG separately to the control treatment and to the CPH meal with contrast analysis, we also found that there was no significant difference (P>0.05) between all CPH-extract treatments and the control, and between the fine extracts of CPH and crude CPH meal. Base on period in totally average of ADG, the contrast analysis showed that there was no significant differences (P=0.051) between day 14th and 28th, 42nd, 56th whether in linear or in quadratic degree.

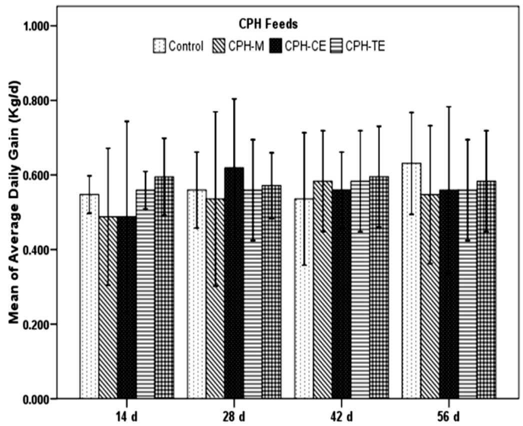

Figure 1. Average daily gain of cattle with different CPH extracts feeds and feeding periods.

Comparing the results with other animals, we indicated that there were some different respond according to ruminant or animal species. Fermented CPH were reported on goats that usage of fermented CPH at the level of 30% had higher (P<0.05) feed intake (560.33g/d), body weight gain (101.79 g/d), and feed conversion (5.50) compared to control treatments with normal feed [6]. Digestibility of dry matter, crude protein, crude fiber, organic matter and gross energy digestibility were significantly higher (P<0.05) in the goats on the control diet than in those on cocoa shell +CPO or cocoa dusk + CPO feeders. In contrast, in the growth rate studies of Djallongke sheep, dry matter intake significantly (*P*<0.01) increased from 73 g/kg $W^{0.75}$ to a maximum of 101g/kg $W^{0.75}$ as the proportion of CPH increased from 0 to 600 g/kg diet, but growth rates (ranging from 37 to 55 g per day) and feed conversion efficiency were not significantly affected by dietary treatments. Research results in broiler chick diet showed that increasing treated cocoa pod husk level up to 100 g kg^{-1} did not significantly reduce growth performance and apparent digestibility of nutrients.

Restriction in utilization of CPH as feeds basically caused by the theobromine, a compound was known as a antintritionnel factor which limits their usage in livestock [2]. However, our research indicated that there was no significant difference in responds between CPH-T and other treatment especially to the control animals. Early investigation on goat [6] with fermented CPH appeared a significant effect on average body gain and the gaining increase positively with the level of CPH in feed.

Daily body gain was an indicator for feed efficiency which the feed deposited physiologically in the body.

Lower body gain may due to low of feed efficiency in feed ingredient. CPH had high lignin concentration and the fermentation process was able to break the lignin into simple compounds, bio-conversion from fungi such us *Phanerochaete chrysosporium* in fermentation changes its structure by breaking down the linkage between lignin and structural carbohydrates (lingo-cellulose complex) by enxime lininase. In this study we used CPH as feed without fermentation but as an extract. The extraction process consist some process which may had the same effect to fermentation. Milling, massing and chemical treatment also worked to break the lignin or fiber into the simply bounds. However, an advantage approach is needed further for deeply evaluation related to this assume.

B. Blood Profilese

1) Plasma aspartate transaminase (AST)

Plasma Aspartate Transaminase (AST) were same (P>0,05) among control, CPH-M, and CPH-C animlas but thus were significantly higer than CPH-T and CPH-P animals. CPH-T had lower AST value than other treatments. AST level accordig to days of treatment also did not show any significant cnganges (Fig. 2).

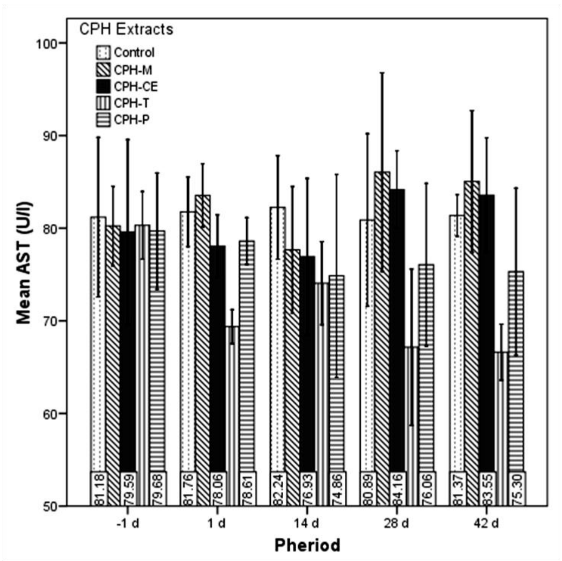

Figure 2. Mean of aspartate transamilase (AST) in blood plasma of bali beef.

Aspartat Transaminase (AST) was one of some indocator to observe liver activiy. The activity of this aminotransferases in blood is very important, the act as a catalyst in connecting the metabolism of amino-acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage [7]. In normal condition according to previous report, AST level on male Bali beef were around 62.93-82.55 UI/l and the differences also found according to the animal ages [8]. The results of our current study was higher than AST blood in lactating dairy cows (57.79 ± 16.49 U/l) in lactation period as was reported by [9], but lower than AST in fattening cattle with tallow and cotton seed oil containing in the diet, with 107.9±5.6 U/l and 112.4±7.4 UI/l respectively; and a wide range of AST levels was reported on early lactation period of Simmental cow (69.46 ± 30.89) [10] and on dairy cows [7]. Control animals appeared the same AST levels as long as days of treatments (P>0.05), also on CPH-M, and CPH-P. Animal responses in CPH-M treatment appear AST average higher whether compared to the other treatment and the days of treatments. The significant changes (P<0.05) were found of CPH-T animals. AST level at pre-treatment was decrease after one day after treatment but a non significant increasing appear at 14th day, however the down line also appeared at 28th and the last of observation day.

Even though all compared literature implies that the utility of CPH dietary in this research were save to the animals, unique evidence as shown on animal with CPH-T would to be concerned further.

2) Plasma urea (blood urea nitrogen)

The Controls had the lowest blood urea nitrogen (BUN) plasma value but was not significantly (P>0.05) different to CPH-C and CPH-P; urea plasma in CPH-M animals was higher than other treatment (P<0.05) except with CPH-T animals. According to the days of treatment, research found, that there was no significant difference between urea level at pre-treatment and one day of treatments, also with after 28 and 52 days. The highest urea levels were found at 14th days of treatment, but the alteration was not significantly different to 28 and 42 days treatments. This means that urea level in blood was more varied after 28 and 42 days of treatment (Fig. 3).

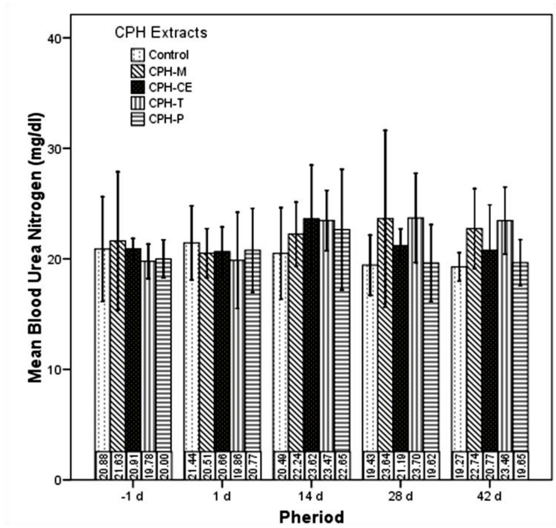

Figure 3. Mean of blood urea nitrogen (BUN) in blood plasma of bali cattle.

Average value of BUN plasma in our research was higher than BUN value were reported (12±6 mg/dl) by Kohar et al [11], that may to imply an increase of protein catabolism or protein digestions, however the values are in normal ranges 32.3±2.4 mg/dl urea or 15.05±1.12 mg/dl of BUN [12]. Increasing of BUN in blood, especially in ruminants may due to un-degradable proteins (UDP) in the diet, increase of ammonia in rumen and had negative effect in energy efficiency or nitrogen utilization for energy. CPH-M animals had higher BUN level (P<0.05) than CPH-C implies that the shape of CPH may effects the concentration of UDP in diet, indirectly it was confirmed from ADG (Fig. 1), which CPH-C animal showed better responds compare than CPH-M animals. Crude or meal shapes and or fermentation process in dietary services may be related with crude fiber concentration in diet. Cocoa shell obtained about 23,3% of crude fiber [13], in other research it obtained higher crude fiber 58.0% in shell and 61.0% in dust. In other hand, lower level of BUN may due to indication for well ammonia utilization in rumen.

Although CPH feed supplement did not appear any significant effect on ADG responds, however, according to AST and BUN parameter indicated an increasing of protein utilization and metabolism, thus, preliminary, theobromine in the CPH diets had no any effects on nutritional absorption. Increasing in protein utilization was followed by non-significant ADG implied that fat forming may restricted through dietary treatment period. There would be wide parameters which need to be observed further.

3) Triglyceride

The triglyceride level on control was roughly the highest (P<0.05) compared to all treatments, while there was no significant differences (P>0.05) within the supplemented CPH feeds.

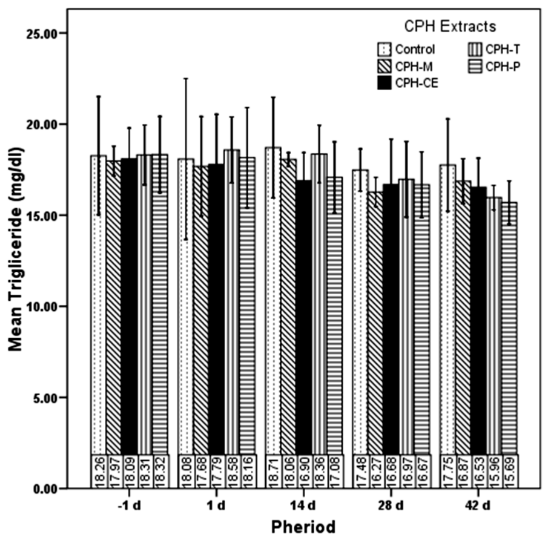

Figure 4. Mean of triglyceride in blood plasma of bali cattle.

Except the controls, all trial animals showed respond that blood triglyceride decrease after 14days treatments, which CPH-CE was the lowest. Finally at the 42 days of treatment, the control animal had higher triglyceride, followed by CPH-M, CPH-CE, CPH-T and CPH-P respectively, however, no significant differences (P>0.05) was found (Fig. 4). Triglyceride levels of Bali beef this research was lower than Hereford and Brahman [14]. As an indicator for Low Density Lipoprotein (LDL) in blood, low level of triglyceride might to imply that compounds which consisted in CPH extract had inhibitory actions in lipogenesis and prevent fat formation in adipose tissue. In other perspective, low of triglyceride level might also indication of triglyceride accumulation in the liver [15]. For further research for this indicator might to be concerned in order to provide any potential effect of CPH-feed and ketosis in ruminants.

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REFERENCES

[1] T. Toharmat, E. Nursasih, R. Nazilah, N. Hotimah, and T. Q. Noerzihad, "Physical characteristic of high fiber feed and its effect on goat consumption and nutrient digestibility," *Media Peternak.*, vol. 29, no. 3, pp. 146-154, 2006.

[2] B. Kouamé, G. Marcel, K. B. André, D. Théodore, *et al.*, "Waste and by-products of cocoa in breeding : Research synthesis," *Int. J. Agron. Agric. Res.*, vol. 1, no. 1, pp. 9-19, 2011.

[3] K. Entwisetle and D. R. Lindsay, Eds., "Strategies to Improve Bali Cattle in Eastern Indonesia," in *Strategies to Improve Bali Cattle in Eastern Indonesia. ACIAR Proceedings No. 110*, 2003.

[4] Haryadi, B. Rahardjo, and D. W. Marseno, "Antioxidant activity of crude polyphenol extract from microwave roasted cocoa beans," *Food Technol. Ind.*, vol. 17, no. 3, pp. 176 - 182, 2006.

[5] G. Freckmann, A. Baumstark, C. Schmid, S. Pleus, M. Link, and C. Haug, "Evaluation of 12 blood glucose monitoring systems for self-testing: system accuracy and measurement reproducibility," *Diabetes Technol. Ther.*, vol. 16, no. 2, pp. 113-22, Feb. 2014.

[6] S. Suparjo, K. G. Wiryawan, E. B. Laconi, and D. Mangunwidjaja, "Goat performance feed with fermented cocoa pod husk," *Media Peternak.*, vol. 34, no. 1, pp. 35-41, Apr. 2011.

[7] F. D. González, R. Muiño, V. Pereira, R. Campos, and J. L. Benedito, "Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows," *J. Vet. Sci.*, vol. 12, no. 3, pp. 251-255, Sep. 2011.

[8] N. S. Dharmawan, I. Bagus, K. Ardana, L. Patologi, K. Veteriner, and F. K. Hewan, "Clinical blood profiles of bali cattle," *J. Vet.*, vol. 13, no. 4, pp. 410-415, 2012.

[9] Z. Stojević, J. Piršljin, S. Milinković-tur, M. Zdelar-tuk, and B. B. Ljubić, "Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period," *Vet. Arh.*, vol. 75, no. 1, pp. 67-73, 2005.

[10] R. Djokovi, V. Kur, Z. Ili, M. Cincovi, M. Petrovi, N. Fratri, and B. Jašovi, "Evaluation of metabolic status in Simmental dairy cows during late pregnancy and early lactation," *Vet. Arh.*, vol. 83, no. 6, pp. 593-602, 2013.

[11] R. A. Kohn, M. M. Dinneen, and E. Russek-Cohen, "Utilization in cattle, sheep, goats, horses, pigs, and rats The online version of this article, along with updated information and services, is located on the World Wide Web at : Using blood urea nitrogen to predict nitrogen excretion and efficienc," *J. Anim. Sci.*, vol. 83, pp. 879-889, 2005.

[12] M. Özdogan, K. Metin, F. Kargin, B. Birincioglu, and A. Önenç, "The effects of diets containing tallow and cotton seed oil on liver and serum parameters in fattening bulls," *Pakistan J. Nutr.*, vol. 5, no. 5, pp. 492-496, 2006.

[13] F. Meffeja, N. Njifutié, Y. Manjeli, T. Dongmo, J. Tchakounté, and R. B. Fombad, "Digestibilité et influence des rations contenant des niveaux croissants de coques de cacao sur les performances des porcs en croissance finition," *Livest. Res. Rural Dev.*, vol. 18, no. 11, p. 165, 2006.

[14] R. Browning and M. L. Leite-Browning, "Comparative stress responses to short transport and related events in hereford and Brahman steers," *J. Anim. Sci.*, vol. 91, no. 2, pp. 957-69, Feb. 2013.

[15] R. M. A. Goselink, J. van Baal, H. C. A. Widjaja, R. A. Dekker, *et al.*, “Effect of rumen-protected choline supplementation on liver and adipose gene expression during the transition period in dairy cattle,” *J. Dairy Sci.*, vol. 96, no. 2, pp. 1102-16, Feb. 2013.

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