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The Effects of Substitution of Corn Meal from the Concentrate of Plus-Complete Feed with Fermented Cocoa Pod on Growth of the Young Male Bali Cattle

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ABSTRACT

Abstract: The study aimed to evaluate the effects of substitution of corn meal from concentrate of plus-complete feed (PCF) with fermented cocoa pod (FCP) on growth of the young male Bali Cattle. Fifteen young male Bali Cattles were randomly assigned into five groups for the substitutions of corn meal from the PCF by FCP treatments i.e 0, 25, 50, 75 and 100 % which each group was replicated by three subgroups. The pods were fermented with yeast *Aspergillus niger* that had been activated by combinations of sugar, urea, NPK fertilizers, and various levels of $ZnSO_4$ and Zn-Cu isoleusinates. The best FCP, that produced NH_3 and VFA in in vitro experiment, was used in the study. The results showed that substitutions of corn meal from the PCF by 100% FCP decreased significantly ($P < 0.01$) Zn and Cu status, energy and N retention, and body weight gain of the young male Bali Cattles. However, the substitutions of corn meal with 0 %, 25% and 50% FCPs, those values did not decrease significant. Despite of corn meal substitutions of PCF by FCP did not decrease significantly plasma glucose and protein consumption, but substitution by FCP between of 0 % and 25 % did not decrease significantly protein consumption. **In conclusion:** the substitution of corn meal from the PCF by the FCP at the level up to 50% can be applied without disturbance the growth of the young male Bali Cattle.

Keywords: $ZnSO_4$, Zn-Cu isoleusinic, cocoa pod, growth, Bali Cattle

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1. Introduction

In previous research, we have found that technological feed formula plus complete feed (PCF) for fattening of Cattle. The PCF contained 150 mg $ZnSO_4 \cdot kg^{-1}$ DM concentrate and 2% Zn-Cu isoleusinates kg^{-1} ration DM diet resulted successful in increasing the weight of Bali Cattle according to their genetic potential i.e 0,721 $kg \cdot d^{-1}$ (Hartati, dkk., 2012a). However, the amount of corn meal in the concentrate component of PCF is quite high at 46.25%. Due to corn is still needed as a food source and also to use in non-ruminant and poultry feed so it is necessary to seek the alternative feed ingredients to replace a part of corn in PCF (Hartati et al., 2010).

Cocoa pod constitute the largest part of the waste cocoa, reaching 70-75.67% (Wong et al., 1986; Darwis et al., 1988). It has been estimated the cocoa pod dry matter production in West Nusa Tenggara and East Nusa Tenggara is around 351,713.96 tons $year^{-1}$. If it is assumed that the cocoa pod to replace the 50% of the forage with a ratio of forage: concentrate 60:40, it can accommodate as many as 120,038.80 ST (Angraeny and Umiyasih, 2008). However, cocoa pod has not been used optimally because they contain low protein (7.17 – 9.36%), high crude fiber (30.16 –

47.87%) and high lignin (27.95 – 38.78%) (Amiroenas, 1990 and Laconi, 1998 Guntoro, et al., 2006, Anggraeny and Umiyasih, 2008), neutral detergent fiber (NDF) and acid detergent fiber (ADF) high (66.3% and 65.1%) and the content of acid detergent lignin (ADL) 28.0% (Smith, 1974 within Sutardi, 1997). Besides, zinc (Zn) and copper (Cu) content of ruminant diets is low respectively 20-38 mg/kg and 8-10 ppm (Little, 1986 and Underwood, 1977), whereas the Zn and Cu very important role in the process of fermentation in the rumen and digestive enzyme activities, absorption and metabolism of nutrients. The low Zn content leads to decreasing of palatability and consumption, microbial and animal growth disturbance, decreasing the activity of the enzyme phosphatase alkaline in calf serum and carboxy peptidase A and B functioning in protein synthesis and absorption of amino acids (Miller et al., 1966; Luecke et al., 1968; Huber and Gershoff, 1973; Bettger et al., 1979 and Hartati, 2008). Trace mineral Zn, Cu, and Se are involved in vitamin metabolism and protein synthesis of animal (Cortinhas et al. 2010 in Cristina et al. 2012). Copper has physiological functions beside related to cellular respiration and heart function it also to bone growth (McDowell, 2003).

Numerous authors as reported everywhere (Hartati 1998 and 2008, Hartati et al., 2009a, Larvor 1993, and Linder 1992) have suggested that enough absorption of Zn into metabolic system could enhance the activity of the enzyme phosphatase alkaline and carboxy peptidase possibly A and B respectively, which it plays an important role in energy metabolism, protein digestibility, absorption of amino acids, energy and N retention. Similarly, it was expected that increasing Cu content derived from the addition of Zn-Cu organic could promote Zn absorption in the post-ruminal, thus increasing the activity of enzymes involved in the synthesis and affecting the increase of body weight gain.

Due to the hard absorption of Cu, Hartati et al. (2012a) have conducted a research to make a high biology value of the Zn-Cu isoleusinolate organometallic compound in a supplement form to make Zn appear at the post rumen. Another study reported in Hartati et al. (2012b) indicated that the quality of the CPF using *Aspergillus niger* activated by sugar, urea, and NPK with the addition of ZnSO₄ and Zn-Cu isoleusinolate undergoing improvement. The PCF obtained from this study was CPF gives the best response to rumen fermentation and digestibility *in vitro*, at level of 15 mg ZnSO₄. L⁻¹ aq and 200 mg Zn-Cu isoleusinolate.L⁻¹ aq. However, FCP that has respond with a best results, empirically used as a substitution of corn from the PCF which has been fed to young male Bali Cattle.

In previous research using the same experimental design (Hartati et al. 2014) studied the effects of substitution of corn meal from PCF by FCP on rumen kinetic, consumption and digestibility nutrient. Results from the research indicated that the treatment effect decreased significantly the consumption and digestibility of dry matter, organic matter, and crude protein. This results showed that treatment without substitution of the corn meal by the FCP at the level of 25% and 50% showed there has no difference in dry matter and organic matter intake. While the crude protein consumption did not decrease significantly between substitution of corn meal from PCF by 0 and 25% FCP. Furthermore, the decreased organic matter and protein digestibility between 0% to both 25% and 50% did not differ significantly, but substitution of corn meal from PCF by FCP between the level of 75% and 100% significantly

decreased. Based on these results it can be showed that the substitution of corn meal from PCF by FCP at the level of 25% and 50% give consumption and digestibility values did not differ significantly; there was no difference between PCF without FCP and substituted PCF by FCP.

The objective of the present study was to evaluate the effects of substitution of corn meal from the PCF by FCP on Zn and Cu absorption, Zn and Cu plasma concentration, protein consumption, energy and N retention, plasma glucose, and body weight gain of the young male Bali Cattle.

2. Materials and Methods

2.1. Materials

The feed used in this study was FCP using fermented *Aspergillus niger* seed as a starter, urea, NPK, ZnSO₄ and Zn-Cu isoleusinolate that offered the best responds to the process of fermentation and digestibility *in vitro*. (Table 1; Hartati et al., 2012b).

2.2. Methods

2.2.1. PCF concentrate preparation

The PCF/plus complete feeds were composed of ammoniation “kume” grass and concentrate containing 150 mg ZnSO₄.kg⁻¹ PCF DM and 2 % Zn-Cu isoleusinolate.kg⁻¹ diet DM. Concentrate in PCF was composed of corn meal, coconut extract, rice bran, fish meal, coconut oil, and salt that contained 17.07 % CP and 78.16 % TDN (Table 1). Concentrate in PCF supplement requires 40% of the dry matter, while the needs of dry matter is determined by 3% of body weight.

Fifteen young male Bali Cattles were used in the study and they were divided into 5 groups and replicated by 3 treatments in a group. The groups were: T0 = 60% PCF + 40% of ammoniation “kume”: (grass and concentrates) without FCP; T1 = 25% PCF as a substitute for corn in FCP; T2 = 50% PCF as a substitute for corn in FCP; T3 = 75% PCF as a substitute for corn in FCP; T4 = 100% PCF without corn in FCP.

2.2.2. Blood collection and sample analysis

Blood sample was taken via jugularis vein and rumen fluid 3 hours after feeding to assess glucosa, Zn and Cu concentration in the blood. Variable measured were protein consumption, blood glucosa, energy and N retention,

Table 1. Composition of concentrate formula as a component of PCF

Ingredients	Composition	Protein (%)	TDN (%)	Protein Concentrate (%)	TDN Concentrate (%)
Corn meal (%)	46.25	10.00	91.00	4.64	42.09
Rice bran (%)	20.50	10.89	66.00	2.23	13.53
Coconut cake (%)	23.00	23.10	74.00	5.31	17.02
Fish meal (%)	8.00	61.20	69.00	4.90	5.52
Lemuru oil (%)	1.50	-	-	-	-
Salt (50	0.25	-	-	-	-
Premix (%)	0.50	-	-	-	-
ZnSO ₄ (mg/kg)*	150.0	-	-	-	-
Zn-Cu isoleusinolate (%/kg)**	2.0	-	-	-	-
Total	100 %			17.07	78.16

Note. Hartati et al. (2009b), *Hartati et al. (2009a), **Hartati et al. (2012a)

Table 2. Composition of substances resulted from cocoa pod fermentation using *Aspergillus niger* and the ration at various treatments

Compositions	PCF	Groups of the treatments				
		T0	T1	T2	T3	T4
Dry matter (%)	92.25	91.03	90.52	91.11	91.29	91.80
Organic matter (%)	83.13	91.72	91.74	91.83	91.22	91.10
Crude Protein (%)	12.18	12.71	11.79	11.10	10.75	10.50
Crude Fiber (%)	30.66	18.81	18.26	17.68	17.41	17.37
Fat (%)	2.85	11.01	10.17	8.83	8.51	8.27
CHO (%)	68.10	67.31	68.56	71.18	72.03	73.03
ADF (%)	57.18	53.13	52.87	52.64	54.27	55.55
NDF (%)	61.36	66.13	65.97	65.73	66.42	66.83
Zn (ppm)	45	47	38.18	37.83	23	26
Cu(ppm)	15.40	6.70	11.70	10.90	9.90	9.40

Sources. Hartati et al. (2014)

Tabel 3. Average-Zn and Cu absorption, Zn and Cu concentration in serum (n = 5)

Zn and Cu status	Groups of the treatments				
	R0	R1	R2	R3	R4
Zn absorption (mg)	138.19±9.15 ^a	149.91±14.97 ^a	148.57±20.78 ^a	104.09±10.59 ^b	89.76±7.83 ^b
Cu absorption (mg)	92.82±5.31 ^a	84.95±4.00 ^a	82.92±1.74 ^a	67.64±8.40 ^c	77.22±3.91 ^b
Zn concentration in serum (mg. L ⁻¹)	0.46±0.25 ^a	0.58±0.10 ^a	0.37±0.17 ^a	0.48±0.42 ^a	0.67±0.32 ^a
Cu concentration in serum (mg. L ⁻¹)	0.29±0.06 ^a	0.24±0.07 ^a	0.27±0.11 ^a	0.21±0.03 ^a	0.21±0.05 ^a

Notes: Means of superscript different letter in the same row indicate significant differences ($P < 0.05$).

and body weight gain of the young male Bali Cattles. Crude protein consumption is the differences between feed crude protein offered and the remaining, Zn and Cu absorbed is the offerrences between in the ration and faeces was determined by using AAS. N retention is the differences between N intake and N in faeces and urine, while energy retention is the differences between energy intake and metabolic energy in the faeces. Weighing of experimental animal was once every two week and there were 5 times of weighing during the work.

2.3. Statistical analysis

Data are expressed as the mean $\pm$ SD of five independent experiments. Statistical evaluation of the data was performed by analysis of variance and the Duncan's multiple range test using SPSS package software Release 17. Statistical significant was considered at $P < 0.05$ or $P < 0.01$.

3. Results and Discussion

Feed used in this study consisted of ammoniation Kume, standinghay grass, and concentrate consisting of corn meal, fine bran, coconut meal, fish meal, coconut oil, salt and premix. Other feed ingredients were used as substitutes cocoa pod was fermented using *Aspergillus niger*, which was activated by the addition of ZnSO₄ and Zn-Cu isoleusinates; the best responds to the process of fermentation and digestibility in vitro were at 15 mg ZnSO₄.L⁻¹ aq and 200 mg Zn-Cu isoleusinates.L⁻¹ aq. Composition results of cocoa pod fermentation using *Aspergillus niger* and the ration at various treatments and Zn and Cu status is showed in Table 2 and 3.

Supplemntation of Zn-Cu isoleusinates on FCP,

which was prepared through biofermentation proses using *Neurospora* sp (yeast) by adding amino acid isoleusinates onto steamed cassava, potentially to increase rumen microorganism growth, especially cellulolytic bacteria which are important in digesting fibre (Hartati et al. 2009a). Data Table 2 showed that the decline in the content of ADF and NDF in the ration containing 25% and 50% PCF substitution of corn, but the decrease did not show significant differences compared to the diet without PCF. While the substitution of 75% and 100% PCF replace corn increased content of NDF and ADF. These conditions can decrease fiber digestibility levels, due to lack of available sources of carbon chain (C) and the availability of N and Zn and Cu were lower, then the optimal microbial protein synthesis and subsequent fermentation in the rumen disturbed.

There has been reported that an enough available content of Zn and Cu in the ration can optimize rumen microorganisms protein synthesis (Hartati et al. 2014), so that it could perform the optimal fermentation process. An increase in the content of Zn and Cu in maize substitution in FCP by 25% and 50% increase PCF even lower, but the enough fat content can increase the absorption of Zn (Table 3). Hartati et al. (1998; 2008 and 2009b) suggested the enough absorption of Zn leads to increase the activity of the enzyme phosphatase alkaline and carboxy peptidase possibly A and B respectively, which plays a role in energy metabolism, protein digestibility, and absorption of amino acids (Larvor, 1993). Cortinhas et al. (2010) reported that the Zn and Cu minerals beside involved in vitamin metabolism and the immune system of the animal, both minerals also

involved in protein synthesis. Similarly, the increasing Cu content derived from the addition of Zn-Cu isoleusinates has been reported to have much absorption in the post-ruminal, thus increasing the activity of enzymes involved in the synthesis and will affect the increase in body weight gain. Data Table 2 clearly show the content of Zn and Cu at the level of substitution of corn by 25% and 50% higher than the substitution level of corn meal by 75% and 100% PCF. Average Zn and Cu absorption and Zn and Cu concentration in plasma on various treatments are shown in Table 3.

Based on the Table 3 data that showed higher Zn absorption in 60% PCF + 40% ammoniation "kume" (grass and concentrates without FCP). Then is followed by the treatments with 25%, 50 %, 75 % PCF as a substitute for corn meal in FCP and lowers in 100% PCF without corn meal replaced by the FCP. The results indicated that treatment highly significantly ($P < 0.01$) decrease Zn and Cu absorption. The decreasing of Zn and Cu absorption not significant on ration substitution of which 0, %, 25 % and 50 % corn meal of PCF by the FCP, while the difference caused by the treatment was significantly affected ($P < 0.05$) to able the absorption of both Zn and Cu consumption. This condition due to the effects of the consumption decrease of the dry matter and organic matter. Rations substituted by PCF at the level of 25% and 50% showed no difference in dry matter intake (Hartati et al. 2014). Similarly, the consumption of organic matter between the ration without substituted and a substituted PCF at the level of 25% and 50% did not differ significantly. Between 50% and 75% rations were also no different, but the lowest consumption of organic matter in the ration was found at the substituted 100% by PCF. There were no differences in dry matter intake and organic matter is likely due to the flow rate of the feed ration without post-rumen and substituted at same level of 50%. Beside due to the Zn consumption, Zn absorption is also because of nabati oils content in PCF as resource of the unsaturated fatty acids used as prostaglandin PgE_2 synthesis precursor to increase Zn absorption (Hartati, 1998 and 2008a). Song and Adham (1979) stressed that PgE_2 was able to increase Zn absorption up to 54 %. Hartati et al. (2012a) has also suggested that Cu supplementation in form of organic compound Zn-Cu isoleusinates with high biological value was able to increase Cu absorption at post rumen and the increase up to 120 % compared to basal diets without supplementation.

The previous study of Hartati (1998 and 2008) and Hartati et al. (2009a; 2012a) that the supplementation 150 mg $\text{ZnSO}_4 \cdot \text{kg}^{-1}$ DM concentrate and 2 % Zn-Cu isoleusinates conducted that the increase of Zn absorption was able to increase enzyme alkaline phosphatase activity which functioned in energy metabolism and carboxy peptidase A and B which functioned in protein digestibility and amino acids absorption, respectively. Thus, energy metabolism and amino acids absorption that leads to increase

energy and N retention, which is in turn able to increase body weight gain of the young male Bali Cattles.

Results of the present experiment indicated that average of protein consumption, N and energy retention, plasma glucose, and body weight gain of the young male Bali Cattles are reported in Table 4. The results showed that substitution of corn meal from PCF by the FCP led to highly significant ($P < 0.01$) decrease protein consumption, N and energy retention, and body weight gain, but not on plasma glucose levels. The decreasing of protein consumption was not significant on ration substitution of which 0, %, 25 % and 50 % corn meal from PCF by FCP, but the difference on ration substitution for the 75 % and 100 % by FCP found significantly ($P < 0.05$). To be effected by the consumption both of Zn and Cu on their absorption, the protein consumption as reflected by the total protein decreasing in plasma was already reported by Lestari et al. (2014) that indicated by the N retention decreased ($P < 0.01$). However, the decreasing N retention in substituted corn meal at the level of 25% and 50% did not differ significantly effected (Table 4). This result is in agreement with our previous work (Hartati, 1998 and 2008, and Hartati et al., 2012a) that amino acids absorption effected by the increase of protein consumption and Zn absorption.

The results indicated that substitution of corn meal from PCF by FCP did not significantly decrease the plasma glucose level. This means that FCP can be used as feeding materials to substitute corn meal of the PCF by FCP at the level of 100%. This result the same as with propionic acids that might be due to the substitution of corn meal in FCP by PCF until level 100% are still capable of doing optimal propionic acids (Hartati, et al. 2014). This result also supported by Bergman et al (1966) and Leng (1967) in Manafe (2009) that 50-54% absorbed propionic acids might be converted to plasma glucose. Consequently, the decreasing propionic acids is due to the decreasing energy metabolism and therefore decrease the energy retention and plasma glucose levels, but in this result the decreasing plasma glucose on feed with substitution corn meal from PCF by the FCP until 75 % did not significant (Table 4).

In this study, the decrease energy and N retention lead to decrease the body weight gain of the young male Bali Cattle. The results have indicated that substitution of corn meal from the PCF by FCP at the level 75 % until 100 % highly significantly ($P < 0.01$) lead to decrease the body weight gain. However, substituted corn meal of the PCF by FCP at the level of 25% and 50% did not differ significantly effected. Similarly, a decrease in protein consumption, N and energy retention between the substitution of 25% to 50% which was found that there is no significant difference. Based on these results, the substitution of corn meal from the PCF by FCP at the level of 25% and 50% give protein consumption, N and energy retention, and plasma glucose levels did not differ significantly.

Table 4. Average protein consumption, N and energy retention, plasma glucose and body weight gain of young male Bali Cattles

Item	Groups of the treatments				
	R0	R1	R2	R3	R4
Protein consumption (g.d ⁻¹)	386.2 ±11.7 ^a	380.3 ±9.0 ^a	368.2± 11.7 ^b	360.1 ±15.7 ^b	339.5±12.7 ^c
N retention (g.d ⁻¹)	40.4 ±1.1 ^a	37.1 ±3.2 ^a	32.3±5.5 ^c	29.3 ±2.6 ^b	25.1 ±1.2 ^d
Energy retention (Kcal.d ⁻¹)	10423.5 ±786.3 ^a	10480.4 ±783.0 ^a	10357.4 ±853.3 ^a	10227.9 ±580.3 ^a	9083.1 ±492.8 ^b
Plasma glucose (mg.dL ⁻¹)	84.36 ±4.36 ^a	83.98±3.32 ^a	83.77±1.44 ^a	82.50±9.66 ^a	81.91±8.87 ^a
Body weight gain (g.d ⁻¹)	269.2±71.4 ^a	265.0±14.8 ^a	235.0±72.9 ^a	192.3±12.3 ^b	76.9±25.6 ^c

Notes: Means of superscript different letter in the same row indicate significant differences ($P < 0.05$).

4. Conclusion

Based on these results, it can be concluded that the substitution of corn meal of PCF by FCP at the level of 25% and 50% give plasma glucose, N and energy retention, and daily body weight gain of the young male Bali Cattles did not differ significantly difference compared with ration intakes between the PCF and the corn meal substitution from the PCF by the CFP. This means that FCP can be used as feeding material to substitute corn meal of the PCF at the content up to 50% level.

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