Supplementation of $ZnSO_4$ and Zn-Cu Isoleucinate in Ration to Improve Growth and Body Immunity of Young Male Bali Cattle

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Abstract. An experiment was conducted to evaluate the supplementation of zinc sulphate and Zn-Cu isoleucinate in the local feeds basal diet of young male Bali cattle. The experimental design used was randomized completely block design. The experimental animal were randomly assigned into four group of treaments diet were formulated as*:* R0 = 60% ammoniated “kume“ grass standinghay + 40% concentrate; R1 = R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 1 % Zn-Cu isoleucinate; R2 = R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 2 % Zn-Cu isoleucinate; R3 = R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 3 % Zn-Cu isoleucinate. The basal diet consisted of ammoniated “kume“ grass standinghay, while concentrate consisted of corn meal, rice bran, coconut cake, fish meal, *lemuru* oil and premix. The crude protein content of basal diet was 17%, while total digestible nutrients (TDN) was 78%. The supplementation of zinc sulphate and and Zn-Cu isoleucinate in the diet did not increase significantly crude protein consumption, zinc and cuprum concentration, N and energi retention. However supplementation zinc sulphate and Zn-Cu isoleucinate increase significantly dry matter, energy, zinc and cuprum consumption, zinc and cuprum absorbtion, phosphatase alkaline, growth and imonoglobulin concentration of young male Bali cattle. The highest daily gain ($0.721.day^{-1}$) was achieved at level of $ZnSO_4$ and Zn-Cu isoleucinate supplementation of 150 mg $ZnSO_4$ kg^{-1} concentrate and 2% Zn-Cu isoleucinate kg^{-1} diet.

Key words: Bali cattle, Zn-SO_4, Zn-Cu isoleucinate, growth, immunoglobulin

Abstrak. Penelitian dilakukan untuk mengevaluasi penambahan seng sulfat dan isoleusinate Zn-Cu dalam pakan lokal basal pada sapi Bali jantan muda. Rancangan percobaan yang digunakan adalah rancangan acak kelompok. Hewan percobaan secara acak dibagi menjadi empat kelompok perlakuan pakan yaitu R0 = 60% rumput “kume” hay teramoniasi + 40% konsentrat, R1 = R0 + 150 mg ZnSO4 kg-1 DM konsentrat + 1% isoleusinate Zn-Cu, R2 = R0 + 150 mg ZnSO4 kg-1 DM konsentrat + 2% isoleusinate Zn-Cu, R3 = R0 + 150 mg konsentrat ZnSO4 kg-1 DM + 3% isoleusinate Zn-Cu. Pakan basal terdiri dari rumput "kume" hay teramoniasi, sedangkan konsentrat terdiri dari tepung jagung, dedak padi, bungkil kelapa, tepung ikan, minyak lemuru dan premix. Kandungan protein kasar pakan basal adalah 17%, sedangkan total nutrisi tercerna (TDN) adalah 78%. Penambahan seng sulfat dan dan isoleusinate Zn-Cu dalam pakan tidak secara signifikan meningkatkan konsumsi protein kasar, seng dan konsentrasi tembaga, N dan retensi energi. Namun penambahan seng sulfat dan isoleusinate Zn-Cu secara signifikan meningkatkan bahan kering, energi, seng dan konsumsi tembaga, penyerapan seng dan tembaga, basa fosfatase, pertumbuhan dan konsentrasi imunoglobulin pada sapi Bali muda jantan. Pertambahan bobot badan harian tertinggi (0.721 kg day-1) yang dicapai pada tingkat penambahan ZnSO4 dan Zn-Cu isoleusinate 150 mg ZnSO4 kg-1 konsentrat dan 2% Zn-Cu isoleusinate kg-1 pakan.

Kata kunci: sapi Bali, Zn-SO4, Zn-Cu isoleusinate, pertumbuhan, imunoglobulin

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Introduction

In East Nusa Tenggara, bali cattle is the most famous commodity and very adaptive to the environment condition and the low quality of feeds (Katipana and Hartati, 2005). Effort to improve productivity of cattle, faced a problem of feed availability, especially three months prior to late dry season when grass production is still high but only available in the form of standinghay. On this condition, the grass quality is very low containing about 88.89%neutral detergent fiber (NDF), 2.56% crude protein, 38.75% crude fiber, and the in vitro digestibility of dry matter and organic matter were 45.86% and 48.69%, respectively, and solubility was 21.89% (Hartati and Katipana, 2006), fat, Zn and Cu were 1.90%, 4.42 $mg.kg^{-1}$ dry matter and 15 $mg.kg^{-1}$, respectively (Hartati et al., 2007; Hartati et al., 2009b).

In attempting to optimize rumen fermentation, nutrient supply to stimulate the growth and activity of rumen microbes is needed. Beside protein and energy content to optimize the synthesis and growth of rumen microbes, Zn and Cu minerals are also needed. In accordance, Thalib et al. (2000) stated that to improve bacteria activity in digesting crude fiber of feeds, it was necessary to provide growth factor substances in the mix form of vitamin and mineral (Cu and Zn). Meanwhile, Durand and Kawashima (1980) reported that zinc was potential as a growth limiting factor for rumen microbes. Zinc is required in high enough amount i.e. 130 – 220 ppm for microbial growth (Hungate, 1966), while zinc requirement for growing ruminants about 40 – 50 ppm (NRC, 1980). To meet the zinc requirement of both rumen microbes and animal, it is not from feeds alone because according to Little (1986) and NAS (1980) zinc content of tropical herbage feedis low namely between 20 to 38 $mg.kg^{-1}$ dry matter.

Hartati et al. (2009b) reported that Zn and Cu content of basal diet consisted of ammoniated kume grass standinghay and concentrate were very low, 6.27 and 9.8 $mg.kg^{-1}$ dry matter, respectively, and Zn availability was far from the requirement for growth of rumen microbial namely 130 – 220 $mg.kg^{-1}$. This low Zn content in the ration lead to microbial growth disturbance. Therefor, in this research, 150 mg $ZnSO4.kg^{-1}$ dry matter was supplemented into the ration as recommended by Hartati et al. (2009a). In addition, the supplementation of Zn-Cu isoleucinate per kg dry matter ration is potentially to increase rumen microbes growth, especially cellulolitic bacteria which are important in digesting fibre. Due to hard to be absorbed of Cu, in this research, a high biology value organic compound Zn-Cu isoleucinate also was supplemented and it was expected will available at post rumen.

Previous work of Hartati et al. (2009b) showed that the supplementation of Zn-Cu isoleucinate (3000 ppm Zn and 500 ppm Cu) which were prepared through biofermentation process using Neurospora sp. (yeast) by adding amino acid isoleucinate onto steamed cassava, which has succeded increase fermentation process and in vitro digestibility. Moreover, reported that combination of supplementation $ZnSO_4$ and Zn-Cu isoleucinate at level 2% was able to optimize production of $N\text{-}NH_3$ and total VFA, in vitro digestibility of both dry matter and organic matter. Those in vitro digestibilty results (Hartati et al., 2009b), therefor, has been biologically tested on young male Bali cattle, to study the response in terms of ration dry matter cunsumption, Zn and Cu absorption, alkaline-phosphatase activity, energy and N retention, immunoglobuline (IgG) content as indicator of level of body immunity and body weight gain. The output of the research is a plus complete feed formula for fatenning i.e. a complete feed supplemented by $ZnSO_4$ and Zn-Cu isoleucinate as the limiting factor for rumen microbial growth, enzyme activity, growth hormone and level of body immunity.

It is expected that the supplementation of by $ZnSO_4$ and Zn-Cu isoleucinate into ration consisted of ammoniated "kume" grass standinghay, is able to optimize fermentation in the rumen that leads to increase the intake of dry matter, Zn, and Cu, activity of alkaline-phosphatase and carboxy-peptidase by chance functioning as protein digestion and amino acid absorption, respectively, and energy metabolism that leads to increase energy and N retention, which is in turn able to increase body weight gain of young male Bali cattle.

Materials and Methods

This research was carried out for three months consisted of two week adaptation period and ten week data collection period. Sixteen heads of young male Bali cattle with an initial body weight of 141.5±20.75 kg (CV = 19.61%) were employed. Basal diet treated consisted of ammoniated ”kume” grass standinghay and concentrate in the ratio of 60 % : 40 %. Concentrate was formulated from local ingredients such as yellow maize, rice bran, coconut cake, fish meal, ”lemuru” fish oil, salt, and premix, containing 17.07 % crude protein and 78.16 % TDN (Table 1).

The experimental design used was completely randomized block design consisted of four treatments and three replications. The treatments offered were supplementation of 150 $mg.kg^{-1}$ $ZnSO_4.kg^{-1}$ dry matter concentrate, and level of Zn-Cu isoleucinate in a concentration of 3000 ppm Zn and 500 ppm Cu as much as 1, 2 dan 3 % $.kg^{-1}$ dry matter ration. Four treatments were arranged as follows: R0 = ammoniated ”kume” grass standinghay + concentrate (60:40), R1= R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 1% Zn-Cu $isoleucinate.kg^{-1}$ dry matter ration, R2 = R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 2% Zn-Cu $isoleucinate.kg^{-1}$ dry matter ration, R3 = R0 + 150 mg $ZnSO_4.kg^{-1}$ DM concentrate + 3% Zn-Cu $isoleucinate.kg^{-1}$ dry matter ration.

The experimental animals were alloted into four group based on body weight as replication. Ammoniated ”kume” grass was offered at ad libitum twice a day after concentrate and supplement consumed at 8 a.m. and 1 p.m., also concentrate containing ”lemuru” fish oil was offered twice a day at 7 a.m. and 12 a.m. Supplementation of $ZnSO_4$ and Zn-Cu isoleucinate was only offered once a day at 7 a.m. together with concentrate, while drinking water was offered ad libitum.

Blood sample was taken via jugular vein 3 hours after feeding to assess Zn and Cu concentration in the blood. Parameters measured were dry matter consumption, Zn and Cu consumption, Zn and Cu absorption, alkaline phosphatase activity, energy and N retention, and body weight gain of young male Bali cattle. Dry matter consumed is the differences between feed dry matter offered and the remaining. Zn and Cu absorbed is the differences between Zn and Cu in the ration and in the faeces. Concentration of Zn and Cu in the ration and faeces was determined by using AAS. Assessment of alkaline phosphatase activity was using manual Sigma Diagnostics Kit No. 104. N retention is the differences between N intake and N in the faeces and urine, while energy retention is the differences between energy intake and metabolic energy in the faeces. Weighing of experimental animals was once every two week and there were 5 times of

Table 1. Composition of concentrate formula

Ingredients	Concentrate Composition (%)	Ingredients		Concentrate	
		CP (%)	TDN (%)	CP (%)	TDN (%)
Yellow maize	46.25	10.00	91.00	4.63	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” fish oil	1.50	-	-	-	-
Salt	0.25	-	-	-	-
Premix	0.50	-	-	-	-
Total				17.07	78.16

Source: Hartati et al. (2009b)

weighing during the work. Data collected was subjected to analysis of variance and then Duncan's multiple range test for further test.

Results and Discussion

The results showed that the supplementation of $ZnSO_4$ and Zn-Cu isoleucinate in local ingredients based diet significantly ($P<0.05$) affected on the intake of dry matter, Zn and Cu, but not on crude protein intake. The highest intake of dry matter, Zn and Cu, was on supplementation of 150 mg $ZnSO_4.kg^{-1}$ dry matter concentrate and 2 % Zn-Cu isoleucinate.kg^{-1} dry matter ration (Table 2).
The different dry matter intake was because of Zn and Cu intake icrease due to supplementation of $ZnSO_4$ and Zn-Cu isoleucinate. In accordance with Hartati et al. (2009b) it assumed that the sufficiency of Zn and Cu for animal and enriched by amino acid isoleucine was able to increase cellulotic bacterial synthesis, resulted in optimum fermentation in the rumen and the increase of dry matter and organic matter digestibility. In consequency, rate of passage is faster and stimulate the animal to consume more ration which lead to increase dry matter intake. Moreover the difference was also caused by the incease of energy consumed, meanwhile there was no difference in total crude protein consumed. According to Villalba dan Provenza (1997) in Manafe et al. (2009) protein or nitrogen content will affect the amount of ration consumed, meanwhile Sentana (2005) stated that animal will stop to consume if it energy requirement has met, although its rumen capacity was not full yet.
The results further indicated that treatment highly significantly ($P<0.01$) affected the absorption of both Zn and Cu. The increasing of Zn absorption was caused by the increasing of Zn consumed. On the other hand, concentrate offered containing 1.5% "lemuru" oil/kg ration dry matter. Hartati (2008) reported that "lemuru" oil containing 21.9% arachidonic acid used as synthesis precursor PgE2 has proven to increase Zn absorption. Song and Adham(1979) stressed that PgE2 was able to increase Zn absorption up to 54%. It was strongly demonstrated that the highest Zn absorbed was found on experimental animal fed on ammoniated "kume" grass standinghay and concentrate supplemented with 150 mg $ZnSO_4.kg^{-1}$ dry matter concentrate and 2% Zn-Cu isoleucinate.kg^{-1} ration (Table 2). The highest Cu absorbed was found on experimental animal fed on ammoniated "kume" grass standinghay and concentrate supplemented with 150 mg $ZnSO_4.kg^{-1}$ dry matter concentrate and 2% Zn-Cu isoleucinate/kg BK ration. This results met the expectancy that Cu supplementation in form of organic compound Zn-Cu isoleucinate with high biologic value was able to increase Cu absorption at post rumen and the increase reached 120 % compared to basal diet without supplementation.

It was expected 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 function in protein synthesis and amino acids absorption, respectively. In other words the increasing of Zn absorption was able to increase the absorption of digested nutrients to increase body weight gain.

Enzyme alkaline phosphatase is an enzyme whose most activity was affected by fat content in ration besides Zn mineral status. One of the indicator of Zn surplus or deficient in animal was by Zn concentration in the serum. Schlegel dan Windisch (2006) stated that the increasing alkaline phosphatase activity indicated that the animal was Zn deficient. This research results showed the increasing Zn absorption very significantly ($P<0.01$) affected the alkaline phosphatase activity in the serum (Table 3). The highest alkaline phosphatase activity was 67.50 ± 4.43 Iu.L^{-1}. Although this result was

Table 2. Means of intake of dry matter, crude protein, Zn and Cu, status of Zn and Cu, and alkaline phosphatase activity on young male Bali cattle

Parameters	Treatment			
	R0	R1	R2	R3
Dry matter intake (kg)	4.83±0.52^{a}	5.24±0.71^{a}	5.94±0.76^{b}	5.59±0.76^{a}
Crude protein intake (kg)	485.79±82.96^{a}	498.51±70.09^{a}	586.04±75.03^{a}	580.58±96.39^{a}
Energy intake (Mcal)	21.43±2.32^{a}	23.25±3.16^{a}	26.41±3.38^{b}	24.84±3.37^{a}
Zn intake (mg)	51.85±6.71^{a}	394.51±57.08^{b}	570.40±73.52^{c}	595.06±114.98^{ca}
Cu intake (mg)	16.86±2.18^{a}	18.58±2.61^{b}	23.19±2.96^{c}	22.40±3.86^{c}
Zn absorption (mg)	32.46±7.34^{a}	123.79±57.44^{b}	224.11±37.05^{b}	192.98±80.43^{b}
Cu absorption (mg)	6.71±3.61^{a}	9.89±1.98^{a}	14.77±1.2^{ab}	11.47±2.90^{ab}
Zn concentration in serum (mg.L^{-1})	3.25±0.04^{a}	3.50± 0.45^{a}	3.11±0183^{a}	3.01±0.15^{a}
Cu concentration in serum (mg.L^{-1})	1.10±0.42^{a}	0.67± 0.17^{a}	0.78±0.13^{a}	0.83±0.15^{a}
Alkaline phosphatase activity in serum (I*u*.L^{1})	27.25±8.06^{a}	67.50± 4.43^{b}	65.25±5.56^{b}	66.00±3.74^{b}

Values bearing different superscripts at the same rows differ significantly (P<0.05)

Tabel 3. Means of body weight gain, energy and N retention, and concentration of immunoglobuline in serum

Parameters	Treatment			
	R0	R1	R2	R3
Initial body weight (kg)	135.63±23.75^{a}	138.5±32.78^{a}	137.00±36.09^{a}	129.63±29.58^{a}
Final body weight (kg)	162.33±21.39^{a}	170.38±29.31^{a}	177.38±39.80^{a}	169.13±31.66^{a}
Body weight gain (kg^{-1}.h^{-1}.d^{-1})	0.4768±0.05^{a}	0.5692±0.06^{b}	0.7210±0.08^{c}	0.7054±0.05^{c}
N retention (kg)	0.0524±0.01^{a}	0.0577±0.01^{a}	0.07±0.01^{a}	0.0638±0.01^{a}
Energy retention (Mcal.kg^{-1})	11.54±2.62^{a}	14.54±1.56^{a}	16.13±2.21^{a}	15.15±1,82^{a}
Immunoglobulin (mg.dl^{-1})	215.75±41.05^{a}	346.25±61.91^{b}	604.30±80.07^{c}	587.47±70.39^{c}

Values bearing different superscripts at the same rows differ significantly (P<0.05)

lower than that of previous study by Hartati (2008) on young Fries Holstein cattle namely 73-153 Iu.L^{-1} and on pregnant Bali cattle ranging between 70.67–105.33 Iu.L^{-1}, it was still within range as reported by Hartati (2009a) and Hartati et al. (2008). This result was however in contrast with the previous work (Hartati, 1998; Hartati,2008; Hartati et al., 2009a). It might be caused by the supplementation of organic mineral in form of Zn-Cu isoleucinate, so Zn in the cell was sufficient to be utilized by various enzymes especially for alkaline phosphatase activity which was optimally reflected by body weight gain of fattened cattle due to a quite high increase of energy retention in body cell components of young male Bali cattle (Table 3, Figure 1). Also, the experimental animals were young, as stated by Girindra (1987) that the activity of alakline phosphatase is decreasing as age increases.

Besides increasing the activity of alkaline phosphatase, the supplementation of $ZnSO_4$ and Zn-Cu isoleucinate was expected can increase the activity of carboxy pepetidase A and B functioning in protein synthesis and absorption of amino acids. N retention in this research has increased. Although Table 3 and Figure 1 showed that the supplementation of ZnSO4 and Zn-Cu isoleucinate on young male Bali cattle fed on ammoniated ”kume” grass standinghay and concentrate containing 1.5% ”lemuru” fish oil per kg ration was not significantly affected the N retention, but showed a significantly enough (P<0.09)

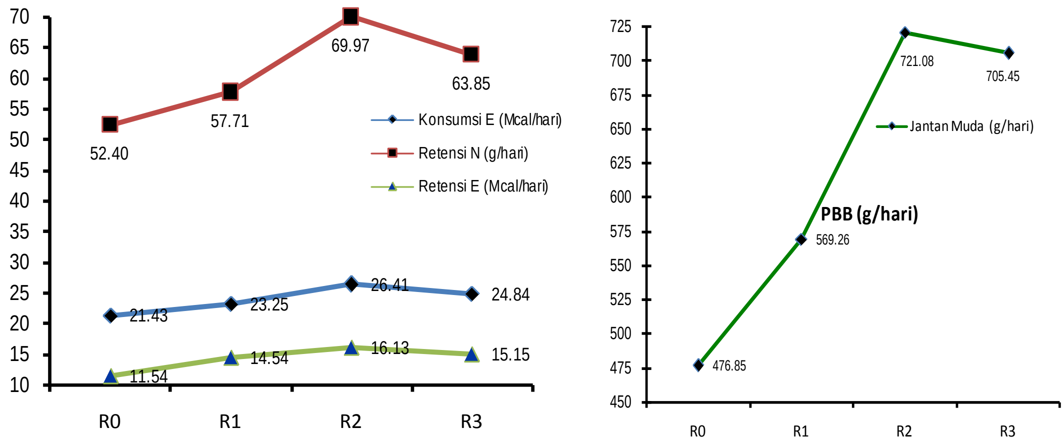

Figure 1. Curve of body weight gain, energy consumption, energy and N retention

increasing of N retention. This is proved that the supplementation of Zn and Cu combination in forms of either organic or in-organic compound has succeded increase enzyme activity which able to improve the usefullness of digested feed for body weight gain of young male Bali cattle. The highest energy and N retention was found on ration R2 i.e. ration supplemented with 150 mg ZnSO4.kg^{-1}drymatter consentrate and 2% Zn-Cu isoleucinate/kg dry matter ration. This increasing energy and N retention was reflected on the highly significant (P<0.01) body weight gain of young male Bali cattle (Table 3), and the highest was 0.7210±0.08 kg d^{-1}. The body weight gain reached in this research was in accordance with genetic potential of young male Bali cattle fattened that is ranging from 0.6-0.7 kg.d^{-1}. If compared to the body weight gain of experimental animal without supplement offered, this gain has increased 51%.

Similarly, the supplementation of $ZnSO_4$.kg^{-1} dry matter and Zn-Cu isoleucinate very significantly (P<0.01) affected the increasing of immunoglobulin (IgG) concentration in serum. The highest increase of immunoglobulin (IgG) was on cattle fed on ammoniated ”kume” grass standinghay and concentrate supplemented with 150 mg $ZnSO_4$.kg^{-1} concentrate and 2% Zn-Cu isoleucinate.kg^{-1} ration dry matte. Table 3 showed that concentration of immunoglobulin (IgG) was ranging from 215.70 – 604.3 mg.dl^{-1}, still in normal ranging that is 16–1890 mg.dl^{-1}, so the animal was able to restrain from disease attack. It showed that all experimental animals in this research were in good healthy, so their physiology functioned well in increasing productivity as reflected on body weight gain as their genetics potential.

Conclusions

Based on the results and discussion above, in attempting to formulate a complete feed formula of local based feed for fattening, it is concluded that (1) the supplementation of 150 mg $ZnSO_4$/kg dry matter concentrate and 2% Zn-Cu alkaline phosphatase significantly increased protein consumption, energy and N retention, and body weight gain of young male Bali cattle. The higest body weight gain of young male Bali cattle namely 0.7210±0.08 kg/d was found on treatment R2: ammoniated ”kume” grass standinghay and concentrate (60%:40%) supplemented with 150 mg $ZnSO_4$/kg dry matter normal range namely 16-1890 mg/dl, enabling the animal to restrain disease attack, (2) a complete feed formula of local based feed for young male Bali cattle fattening, supplemented with 150 mg $ZnSO_4$/kg dry matter concetrate and 2% Zn-Cu isoleucinate/kg dry matter ration able to increase body weight gain as much as 51% was higher than that of ration without supplementation.

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