Original Research Article

Effect of Supplementation of Concentrate containing $ZnSO_4$ and Zn-Cu isoleucinate on Growth and Post Partum Reproductive Performance of Bali Cows Grazing Native Patures

Comment [MF1]: Pastures

ABSTRACT

Aims: This research was carried out to evaluate effect of supplementation of ZnSO4 and Zn-Cu isoleucinate into Complete Feed Plus (CFP) on the growth in the last period of pregnancy and the post partum reproduction efficiency of Bali cows reared semi-intensively.
Study design: The experiment employed twentyseven Bali cows at the last period of pregnancy. The animals were grouped into three treatment groups consisted of grazingon the nearby pasture without supplement (R0), and supplemented with either Gliricidia and Leucaena leaves (R1), or with concentrate supplement containing 150 mg ZnSO4 kg^{-1} PCF DM and 2% Zn-Cu isoleucinate kg-1 diet DM (R2). Data collected was then analyzed using analysis of variance and then Duncan's multiple range test for further test by using SPSS package Release 19.
Results: The effect of basal diet supplemented with PCF containing 150 mg ZnSO4.kg-1 DM PCF and 2% Zn-Cu isoleucinate.kg-1 DM diet was very significantly (P<0,01) increased the the propionate production, N-NH3 production, plasma glucosa, N and energy retention, daily gain of Bali cows in the last period of pregnancy, calf birth weight, estrous cycle and estrous post partumaccelaration, and body weight of weaned calves. Average daily gain of Bali cows in the last period of pregnancy, calf birth weight, body weight of weaned calves, estrous cycle and post partum estrous were found on feed supplement treatment containing 150 mg ZnSO4 kg-1 DM of PCF and 2% Zn-Cu isoleucinate kg-1 DM of diet.
Conclusion: Overall, the results of this work showed that basal diet supplemented with PCF containing 150 mg ZnSO4.kg-1 DM PCF and 2% Zn-Cu isoleucinate.kg-1 DM diet had improved fetus growth of Bali cows in the last period of pregnancy which was indicated by the higher calf birth weight compared to without PCF supplemented, and also improved postpartum reproduction efficiency on cows that reared semi-intensively.

Comment [MF2]: delete

Comment [MF3]: glucose

Comment [MF4]: acceleration

Keywords: ZnSO4, Zn-Cu isoleucinate, semi intensively, Bali cows, post partum estrous

1. INTRODUCTION

Beside dietary protein and energy content, Zinc (Zn) and Copper (Cu) are also required to optimize rumen microbial protein synthesis and the growth of ruminant animals. Zn is essential for ruminants since it has important role in a number of biochemical functionand its

deficiency can affect growth, reproduction, immune system and gene expression in ruminants [1]. A level of 130-220 ppm of Zn is needed for microbial growth and about 40-50 ppm for growing of the animals[2] .Beside Zn, ruminant animals also need Cu for various enzyme function [3]. Cu deficiency is known to decrease growth [4] and body immunity [5]. Durand and Kawashima [6] (1980) reported that both Zn and Cu were potentially growth limiting factor for rumen micro-organism in cattle consuming low quality roughage. In East Nusa Tenggara Province of Indonesia, grass on the pasture contains very low amount of both Zn and Cuparticularly during the dry season. Pasture grasses in the area commonly contain high fibre (88,9% NDF) and low crude protein (2.56% CP) with zinc (Zn) and Copper (Cu) contents of 4,42 mg.kg-1 DM and 15 mg.kg-1 DM respectively [7]. Therefore, providing supplemental feeds containing Zn may improve the performance of grazing cattle in theprovince.

Several experiments have been conducted on the supplementation of ZnSO4 and Zn-Cu isoleucinate in Bali cattle. It was shown from our previous study that the growth rate of young male Bali cattle was improved by supplementation of those micro minerals with the highest improvement was recorded with the supplementation of concentrate containing 150 mg ZnSO4.kg-1 DM and 2% Zn-Cu isoleusinate[8]. This positive effect of the supplementation ZnSO4 and Zn-Cu isoleucinate was due to the increase of growth and immunoglobulin (IgG) concentration as an indicator of the level of body immunity of the animals [8]. However, the benefits of supplementing $ZnSO_4$ and Zn-Cu isoleucinate on grazing cows particularly their pre-and postpartum reproductive performance has been largely unknown. Therefore, the objective of this experiment was to investigate the effect of supplementing a concentrate containing ZnSO4 and Zn-Cu isoleucinate on the performance at the last period of pregnancy and the post partum reproductive performance of grazing Balinative pasture.

Comment [MF5]: Post-partum

Comment [MF6]: Post-partum

2. MATERIAL AND METHODS

2.1 Animals and Experimental Design

This research was carried out in the Province of East Nusa Tenggara (ENT), Indonesia, for six months consisted of two weeks adaptation period and ten weeks of data collection. As many as 27 Bali cows at the last three-mester of pregnancy were involved in the present experiment following a randomized block design with three treatments and 9 replications. Those cows were grouped into three groups of nine cows based on their live weight. Three cows in each group were assigned to the treatments, i.e. grazing the nearby native pastures without supplements as control (R0) or supplemented either with gliricidia (*Gliricidasepium*) and leucaena (*Leucaenaleucocephala*) leaves (R1) or a concentrate feed containing 150 mg $ZnSO4.kg^{-1}$ CFP DM and 2 % Zn-Cu isoleusinate (3000 ppm Zn and 500 ppm Cu).kg^{-1} concentrate at DM bases (R2). The preparation of organic Zn-Cu isoleucinate was following the method described by Hartati et al. [7]. The concentrate was composed of corn meal, coconut cake, rice bran, fish meal, coconut oil and salt to contain 17.07 % CP and 78.16 % TDN (Table 1).

Comment [MF7]: of adaptation

Table1. Chemical composition of the concentrate *)

Feed stuffs (FS)	Composition (%)	PS CP (%)	PS TDN (%)	Concentrate CP (%)	ConcentrateTDN (%)
Corn meal	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

Comment [MF8]: , or .

Fish meal	8,00	61,20	69,00	4,90	5,52
Coconut oil	1,50	-	-	-	-
Salt	0,25	-	-	-	-
Premix	0,50	-	-	-	-
Total				17,07	78,16

(*) [6].

The supplements were given at a level to provide approximately 40% of the estimated DM intake at 3% of body weight. The animals were offered the supplements once a day in the morning before the cows were released for grazing. Drinking water was available *ad libitum*on the pasture.

2.1 Variables and Measurements

Blood sample sample was taken via jugularis vein three hours after feeding. Blood sample was immediately centrifuged at 3000 rpm for 15 min to separate plasma from the cells and immediately frozen before analyzed for plasma glucose concentration following the procedure using Spectrophotometry. At similar day, following the blood sampling, rumen fluid was collected through orophangeal tube connected to a vacuum pump. The collected rumen fluid was strained with three layers of cheese cloth and the pH of rumen liquid was immediately assessed for pH. The strained liquid was then frozen before the analyses for NH3 and partial VFA concentration. Assement of N-Amonia (NH3) concentration was conducted by using Conway microdiffusion method (General Laboratory Procedure, 1966). Meanwhile, Gas Chromatography was employed to determine the concentration of acetate, propionate and butyrate.

Variables measured were growth at the last period of pregnant i.e. daily cows body weight gain and fetus growth, calf birth weight and efficiency reproduction i.e. estrous cycles, estrous post-partum, and weaning weight. Zn and Cu absorbed is the differences between in the ration and feces. Parameters measured in this experiment were both nutrients consumption and digestibility based on method described by Ranjhan [9]. Indicator consumption and digestibility of dry matter is used chromic oxide (Cr2O3) technique. After 24 hours of Cr2O3 distributed to cows, samples of feces of cows were collected and separated within 24 hours. Total feces samples were also used to analyze nutrient contents in the feces. The formulas used as followed:

$$\text{Dry matter of feces (g/day)} = \frac{\sum \text{Cr2O3 intake daily}}{\text{g Cr2O3 per g of feces}}$$

$$\text{Dry matter feed digestibility (\%)} = 100 - \left(100 \frac{\text{\% indicator in feed}}{\text{\% indicator in feces}} x \frac{(\text{\% dry matter in feces})}{\text{\% dry matter in feed}} \right)$$

$$\text{Dry matter intake (kg/hour)} = \frac{\text{fecal output of dry matter}}{\text{Indigestibility}} x100$$

Zn absoption (mg. day^{-1}) = Zn consumption (mg) – Zn in feses (mg)

Cu absoption (mg. day^{-1}) = Cu consumption (mg) – Cu in feses (mg)

Comment [MF9]: deleted

Comment [MF10]: jugular

Comment [MF11]: oropharyngeal

Comment [MF12]: assessment

Comment [MF13]: Ammonia-N

Comment [MF14]: NH3-N

Comment [MF15]: Chromic oxide (Cr2O3) technique was used to estimate dry mattet intake and digestibility

Comment [MF16]: feces

Comment [MF17]: feces

N retention is the differences between N intake and N in feces and urine, while energy retention in the differences between energy intake and metabolic energy in the feces. Weighing of experimental animal was conducted once every two weeksduring pregnancy and post-partum.

2.1 Statistical Analyses

Data collected was subjected to analysis of variance and then Duncan's multiple range test for further test by using SPSS package Release 19.

3. RESULTS AND DISCUSSION

3.1 Volatile Fatty Acid, Ammonia, Plasma Glucose and Body Weight Gain

Rumen pH and the concentration of N-NH_3 and individual VFAs were shown in Table 2. Results showed that rumen pH was higher in the supplemented cows than that in the control grazing cows. Similarly, the concentration of rumen ammonia significantly increased with the supplementation of concentrate containing 150 mg ZnSO4.kg^{-1} CFP DM and 2% Zn-Cu isoleucinate. kg^{-1} of ration DM but not with the supplementation of Gliricidia and Leucaena leaves.

Comment [MF18]: NH3-N

Table 2. The average of pH, N-NH3 and VFA partial concentration in rumen fluid of Bali cows grazing native pasture supplemented with legumes or concentrate containing ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate

Comment [MF19]: NH3-N

Item	Treatment		
	R_0	R_1	R_2
pH	6.61± 0.72^{a}	6.75± 0.19^{ab}	6.80± 0.64^{b}
N-NH_3 (mM)	3,54±0,71^{a}	4,59±0,01^{a}	6,76±1,11^{b}
VFA:			
Asetate (C2) (mM)	16.46±6.49^{a}	7.23±2.01^{b}	6.53±2.03^{b}
Propionate (C3) (mM)	2.94±1.71^{a}	3.29±1.05^{a}	7.95±1.12^{b}
Iso butyrate (i C4) (mM)	0.73±0.10^{a}	0.52±0.11^{b}	0.18±0.04^{c}
n Butyrate (n butyrate) (mM)	6.39±2.46^{a}	3.38±1.75^{b}	0.70±0.11^{c}

Different Superscripts in the rows show the highly significant difference (P<0.01).

Comment [MF20]: NH3-N

Comment [MF21]: Acetate

Result of the present experiment showed that there was a highly significant difference in the rumen pH change between Bali cows fed with CFP containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM and those who were not. This shows that there is no increase of the rumen fluid acidity as indicated by VFA concentration (Table 2).This result is consistent with our previous study from young male Bali cattle fed with the same basal diet containing 150 mg ZnSO4.kg-1 of CFP DM and 2 % Zn-Cu isoleucinate kg-1 of ration DM [10]. In other word, pH of rumen fluid around 6.0-7.3 is still in tolerable level for rumen micro-organisms growth in supporting biofermentation inside the rumen [11].

Comment [MF22]: In the control group

Comment [MF23]: fermentation

Supplying the raised semi-intensive Bali cows of the last pregnant periode with CFP containing coconut oil 1.5% diet DM (Table 1) and 150 mg ZnSO4.kg-1 of CFP DM and 2 % Zn-Cu isoleucinate. kg-1 of DM ration resulted in significant difference of the increasing N-NH3 in the rumen. This may as a result of increasing of micro-organism synthesis and of decreasing of lyses bacteria role in the rumen. The addition of coconut oil in the supplement might increase the syntheses of micro-organism and thus decrease the lyses of bacteria in the rumen. Interestingly, this condition did not happen on the cows that were fed only with CFP containing ZnSO4 and Zn-Cu isoleucinate. It was assumed that even in order to maintain the increase of body weight gain from the cows raised semi-intensive during dry season, it is important to supply the cows with CFP that containing 150 mg ZnSO4.kg-1 of CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM. The CFP supplementation containing 150 mg ZnSO4.kg-1 of CFP DM and 2% Zn-Cu isoleucinate seems to be very important for giving the effect of optimal body weight gain as Zn is one of the limiting factors for rumen micro-organism growth [6].

Other substrates required for rumen micro-organism growth are ammonia and readily available carbohydrate (RAC). Satter and Slyter[12] recommended 5-8 mM N-NH3/L in rumen fluid that is required for maximum micro-organisms' growth. The average of N-NH3 found in this study was around 6.7 mM N-NH3/L. The higher content of N-NH3 in this study is higher than previous study of Hartati [13] due to the higher Zn content and the age of cows used in this study that allow an optimum microbial synthesis.

Volatile Fatty Acids (VFA) content is an indicator of feeds fermentation ability in the rumen as it is an energy source for microbial activities in the rumen of ruminants. In this study supplementation with 150 mg ZnSO4.kg-1 of CFP DM and 2 % Zn-Cu isoleucinate resulted in highly significant difference on partial VFA (acetate, propionate, iso butyrate, n butyrate) concentration at the last period of pregnant Bali cows. It indicates that substrates for normal fermentation as 69-120 mM VFA in rumen fluid available are fulfilled. Readily available carbohydrates and 3-4% lower than 10% fat supplied may be in the tolerable range to support the condition [14].

As well, there are highly significant differences in VFA (acetate, propionate , isodan n butyrate) profile, and concentration of acetate and propionate in rumen fluid by supplying CFP which was supplemented with 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM. Increasing crude fibre digestibility is supposed to be the supporting factor for these improvements. The high availability of NFE (46,25%) in corn meal may also be involved factor. Increasing propionate concentration is profitable for the host as it is the main precursor for glucose formation. It is indicated by higher glucose concentration in the blood of the treated compared to the untreated cows. Rastogi[15] stated that >50% of glucose for physiological functions of the ruminants are propionate products.

Beside causing disturbance on the fermentation process of the rumen, the deficiency of Cu can lead to the decrease of growth acceleration and immune function in animal [4,5]. Therefore, optimizing rumen fermentation is needed for providing adequate nutrient supply to stimulate the growth and the activity of rumen micro-organism. The availability of biological value of micro mineral Zn in Zn-organic is higher than Zn inorganic [16]. It is also found that biological value of the availability of micromineral Cu in Cu-proteinate is higher than CuSO4 [17]. Kincaid et al. [17] and Schell and Kornegay[16] showed that Zn-Cu proteinate and Zn-Cu isoleucinatecan not be fermented by rumen micro-organism, but it can be hydrolyzed by pepsin. That mean Zn-Cu proteinate or Zn-Cu isoleucinateavailability in pasca rumen show that increased plasma Cu concentration.

Comment [MF24]: period

Comment [MF25]: NH3-N

Comment [MF26]: Do you mean increase proliferation of micro-organisms?

Comment [MF27]: in

Comment [MF28]: study,

Comment [MF29]: indiviual

Comment [MF30]: ?

Comment [MF31]: bioavailability

Comment [MF32]: ?

Comment [MF33]: In another word,

Comment [MF34]: bioavailability

Comment [MF35]: unclear!

As shown in Table 3, there was a highly significant (P<0.01) increase in body weight gain and fetus growth in the supplemented cows with CFP which 150 mg ZnSO4.kg-1 of CFP DM and 2% Zn-Cu isoleucinate kg-1 resulted a higher increase compared to gliricidia and Leucaena leaves. Similar trend was observed for blood glucose concentration, Cu and Zn absorption. Calf birth weight, on the other hand, was only improved with the concentrate supplementation.

In this experiment that there is a highly significant increasing body weight gain and fetus growth at the last period of pregnant Bali cows, and the highest which supplying 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM in CFP compared to basal diet without supplementation. Improving body weight gain Bali cows and fetus growth in this treatment may contribute to increasing Zn and Cu absorbtion (Tabel 3), This study was consistent with in young male Bali cattle as reported by Hartati et al., [8]that supplying increased Zn absorption which may support various enzymes activities especially alkaline phosphatase. The availability of glucose as energy source for fetus growth is significant higher of cows fed CFP containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM than non fed Bali cows last pregnant. This is useful for increasing metabolizable nutrients which 80% of them are carried out to fetus development and growth and resulting in high birth weight. Also, that supplying to improve peptidase activity in both protein metabolism and amino as shown in Table 3.

Beside the above profits, also it is assumed that prostaglandin E2 (PgE2) concentration increased especially by supplying 1.5 % coconut oil as substitution of lemuru oil that could increase Zn absorption as reported Hartati [13] and Song and Adham[18]. The increasing Zn absorption is able to increase alkaline phosphatase activities function in energi metabolism. Beside increasing the activity of alkaline phosphatase, the supplementation of ZnSO4 and Zn-Cu isoleucinate was expected to increase the activity of carboxy peptidase A and B functioning in protein synthesis and absorption of amino acids such as reported byHartati et al. [8].

Table 3. Average body weight gain and fetus growth at the last period of pregnant and birth weight, glucose concentration of cows raised semi intensive of each treatment

Item	Treatment		
	R0	R1	R2
Daily cows body weight gain and fetus growth ($g.d^{-1}$)	142,3±0,21[a]	289,5±0,019[b]	472,3±0,06[c]
Calf Birth weight (kg)	11,13±1,17[a]	12,14±0,85[a]	14,19±1,00[b]
Blood glucose concentration ($mg.dL^{-1}$)	90,50±0,72[a]	92,91±1,56[b]	94,61±0,64[c]
Zn absorption (ppm)	40.28 ± 4.68 [a]	47.31± 2.71 [b]	77.19 ± 1.39 [c]
Cu absorption (ppm)	26.79 ± 3.01 [a]	30.19 ± 1.96 [b]	38.93 ± 2.19 [c]

Different Superscript in the same row means highly significant difference (*P*<0.01)

The increasing of Zn and Cu absorption in Table 3 shows that there was a highly significant difference between the last period of pregnant Bali cows fed with CFP containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM in CFP compared to those which we not fed with the supplementation. This result is consistent with our previous study that Cu supplementation in the form of organic compound Zn-Cu isoleucinate with higher biological value was able to increase Cu absorption at post rumen up to 120 %

Comment [MF36]: delete

Comment [MF37]: weight gain was

Comment [MF38]: delete

Comment [MF39]: associated with

Comment [MF40]: absorption

Comment [MF41]: delete,

Comment [MF42]: significantly

Comment [MF43]: Do you mean unsupplementrd animal?

Comment [MF44]: ?

Comment [MF45]: ?

Comment [MF46]: energy

Comment [MF47]: delet

Comment [MF48]: Mean

compared to basal diet without supplementation [8]. The higher level of Zn and Cu absorption have been known to influence the body weight gain of the cows as a result of the increase of energy and N retention. The increase of energy and N retention is strongly correlated with the highly significant increasing of body weight gain up to 51% of young male Bali cattle compared to the animal without supplementation containing ZnSO4 and Zn-Cu isoleucinate. The higher level of energy was probably due to the increase activity of alkaline phosphatase as the higher level of alkaline phosphatase (either in blood serum or in the cells) has been known to plays an important role in energy metabolism and thus resulting in a high significant increasing of energy retain from the cows. The higher level of energy retain is then useful for body weight gain of the Bali cows and for fetus growth in an intensive system [13].

The same trend for body weight gain was also observed for the last period of pregnant Bali cows supplied with the supplementation of 150 mg ZnSO4kg-1 of CFP DM and 2 % Zn-Cu isoleucinate. kg-1 of ration DM in CFP. Treatment of pregnant Bali cows raised semi intensive with above supplementation had resulted in a higher level of body weight gain of the cows and also to the calves' birth weight. The daily body weight gain of Bali cows treated with the supplementation were higher up to 56 % compared to the Bali cows without supplementation. This might be due to Zn retention and protein utilization was increased by ZnSO4 and Zn-Cu isoleucinate supplementation as also proposed by Salama et al. [19] that Zn retention tended to increase in supplementation containing Zn-Methionin in goats. It was also found that supplementation containing Zn-Methionin improved Zn retention and protein utilization. This result was also supported by research from Jia et al. [20] which indicated that supplementation of 20 mg Zn.kg-1 DM either as ZnSO4 or Zn-Methionin in basal diet containing 23.3 mg Zn.kg-1 DM significantly increased average daily gain in Cashmere goat. The increase of Zn absorption and body weight gain due to the supplementation of additional Zn was also supported by the previous study of [21]. It was reported that the growth of heifer fed with agro-industrial by-product based rations was faster than that received conventional ration. Interestingly, the highest growth of heifers treated with conventional feed was found in those supplemented with Zn-Cu proteinate. Feeding the last period of pregnant Bali cows with the supplementation containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM in CFP have affected significantly the increase weaning of weight, estrous cycle and estrous post- partum. The highest effect was found in Bali cows treated with CFP consisted of 150 mg ZnSO4.kg-1 of CFP DM and 2% Zn-Cu isoleucinate . kg-1 of ration DM. The increase of weaning weight is probably due to the holding of lactation from the cows that were given CFP containing with ZnSO4 and Zn-Cu isoleucinate as their milk provides good enough nutrients that in addition to readily available of carbohydrates and proteins. The presence of available Zn due to the supplementation is very beneficial to increase the digestibility and absorption of amino acids while the availability of Cu tends to increase the role of growth hormone. This is because diet containing 1.5% coconut oil may increase concentrations of serum PgE2 [13]. Besides influenced by the concentrations of serum PgE2, the absorption of Zn is also influenced by the consumption of Zn. Therefore, the addition of Zn can increase the Zn absorption which in turn can increase various range of activity of the enzyme such as carboxy peptidase.

3.1 Weaning Weight, Post Partum Reproductive Performance, Blood Glucose Concentration and Nitrogen and Energy Retention

The average weaning weights, post partum anestrous interval (PPAI) and estrous cycle of cows raised semi-intensively were presented in Table 4. It was found that the calf born from

the dam maintained semi-intensively and fed with CFP containing ZnSO4 and Zn-Cu isoleucinate supplementation had the highest (P<0.05) weaning weight, while the lowest weight was recorded in the calves of the control cows. PPPAI and estrus cycle were significantly shortened with supplementation particularly with more effect of the supplementation of CFP containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM than that of gliricidia and leucaena leaves.

Table 4. Average body weight gain, weaning weight, blood glucose and Hb concentration, N and energy retention of post partum

Item	Treatment		
	R0	R1	R2
Daily cows body weight gain post partum ($g.d^{-1}$)	171,4±0,02[a]	266,0±0,05[b]	376,2±0,02[c]
Weaning weight (kg)	44,98 ± 2,45[a]	63,27 ± 2,17[b]	85,51 ± 5,72[c]
Estrous Cycle (day)	22,5 ± 0,50[a]	21,0 ± 0,0[b]	21,0 ± 0,0[b]
Postpartum Anestrus interval (month)	5,58 ± 0,51[a]	4,57 ± 0,53[b]	4,22 ± 0,44[b]
Blood glucose concentration ($mg.dL^{-1}$)	83,98 ± 0,56[a]	85,16 ± 0,49[b]	86,79 ± 0,56[c]
Blood Hb ($mg.dl^{-1}$)	13,06 ± 0,46[a]	13,66 ± 0,52[b]	14,78 ± 0,69[c]
N retention (g)	7.14 ± 1.59[a]	33.55 ± 8.12[b]	59.94 ± 8.74[c]
Energy retention (kcal)	6.96 ± 0.59[a]	8.99 ± 1.76[b]	14.24 ± 1.76[c]

Different Superscript in the same row means significant difference (*P*<0.05)

The results also showed that blood concentration of glucose and hemoglobin as well as the nitrogen and energy retention increased in the supplemented cows with CFP containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate kg-1 of ration DM produced the better improvement than gliricidia and leucaena leaves.

The needs of basic living nutrients from the cows after giving birth was still available even already used for milk production and the development of reproductive organ reflected from estrous post-partum and estrous cycle. It is interesting to note that the treatments can also accelerate estrous cycle and estrous postpartum cycle. Appropriate estrous cycle in the post partum Bali cows raised semi intensive with sufficient nutrients is observed between 17-24 days or in the average of 21 days postpartum. Similarly, supplementary feeding with Gliricidia and Leucaena leaves, as well as CFP containing 150 mg ZnSO4.kg-1 of CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM was able to accelerate post partum estrous up to 18.10 and 24.37 % compared to post partum estrous cycle from the cows without supplementation which was about 5.58 months (Table 4). This result was also supported by Belli et al. [22] for Bali cows without supplementation with post partum estrous observed around 6.26 months.

Another interesting effect of the treatment with 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate.kg-1 of ration DM was the presence of blood glucose levels of postpartum Bali cows raised semi intensive. The availability of higher blood glucose level makes it flow directly into the breast milk which in turn used as a source of energy for the calf. The results showed that the parent glucose levels was observed higher in Bali cows fed with CFP

containing 150 mg ZnSO4.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM, compared with other treatments. Glucose is used as an energy source for metabolism of nutrients in the cells of which 80% flow into the breast milk resulting in a higher weaning weight. One factor that caused the increase weaning weight was the higher level of the activity of alkaline phosphatase functioning in energy metabolism. Besides that, the supplementation with ZnSO4 and Zn-Cu isoleucinate was able to increase the activity of carboxy peptidase A and B which is important for the protein synthesis, digestion and absorption of amino acids which is then manifested in the highly significant increase of N retention (Table 4). In other words, the higher accumulation of protein or amino acids and energy the higher weaning weight gain. The results of this study confirmed the results found in the previous study [7,13] that the absorption of amino acids influence the intake of protein, the amount of rumen micro-organisms, while the absorption of Zn influence N and energy retention and resulted in higher body weight gain and birth weight. The results of this experiment supported by was in concordance with the study of Andrea et al. [23] which found that partial substitution of Zn, Cu and Mnsulphates with organic trace mineral during the dry phase and lactation period resulted in higher colostrum immunoglobulins and milk fat as well lower calf mortality.

4. CONCLUSION

Supplementation of complete feed plus (CFP) contain 150 mg ZnSO4.kg-1 DM of CFP and 2% Zn-Cu isoleucinate.kg-1 DM of ration increase the ammonia (NH3) concentration, improve the partial VFA concentration that support propionate formation which plays role as precursor in increasing plasma glucose, increase the daily body weight gain of Bali cows at the last period of pregnancy up to 56 % higher compared to the cows fed without supplement, optimize response to plasma glucose, increased weaning weight of Bali calves by 90 % compared to those without supplement offered (the increased weight of 35 % from the parent supplied with Glicidia and Leucaena leaves), acceleration of estrous cycle by 7 % from 22.5 days to 21 days which is a normal condition for Bali cattle and return of time shortened estrous period post partum by 24.37 % from 5.58 months to 4.22 months post partum. Overall, the recent supplementation containing 150 mg ZnSO4.kg-1 DM of CFP and 2% Zn-Cu isoleucinate.kg-1 DM of ration were able to improve the growth of the last period of pregnant and the efficiency of reproduction from post partum Bali cows that raised semi-intensive.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.The experiment has been examined and approved by the appropriate ethics committee.

REFERENCES

1. Underwood, E.J and N.F.Shuttle. 1999. The Mineral Nutrition of livestock, 3nd ed. CABI Publ. Wallingford, Oxon, U.K..
2. National Academy of Sciences (NAS). Mineral Tolerance of Domestic Animals. Washington, D.C.
3. Underwood E.J. 1977. Trace Elemen in Huma and Animal Nutrition. 4 th Ed. New York. Academic Press.

4. Mills, C.F., A. Dalgarnodan G. Wenham. 1976 Biochemical and pathological changes in tissue of freicisian cattle during the experimental induction of copper efficiency. Br. Nutr. 35: 309.
5. Suttle, N.F. and D.G.Jones. 1985. Copper and disease resistence in sheep: A rare natural confirmation of interaction between a spcific nutrient and infection. Proc. Nutr. Soc. 45:317
6. Durand M, Kawashima R,. Influence of Mineral in the Rumen Microbial Digestion, In: Y. Ruckebusch and P. Thivend (Edit). Digestive Physiology and Metabolism in Ruminants, 1980.AVI. Publishing Company, Inc. Connecticut.
7. Hartati, E, N.G.F. Katipana and A. Saleh. 2009. Penambahansengpadapakanpadatgizimengandungminyaklemuruuntukmeningkatkanpertumbuhandanberatlahirsapi Bali. Journal Animal Production. 11 (1): 59-65.
8. Hartati, E, A. Saleh, E.D. Sulistijo and J.J.A. Ratuwaloe. 2012. Supplementation of ZnSO4 and Zn-Cu isoleusinate in ration to improve growth and body immunity of young male Bali cattle. Journal Animal Production. 14 (3): 180-186.
9. Rajhan, S.K. 1988. Animal nutrition in Tropic, Edition Vicas Publishing, House, P and T Ltd, New Delhi
10. Erna Hartati, Ahmad Salehdan Edi DjokoSulistijo. 2016. Efeksupplemenatasi ZnSO4 dan Zn-Cu isoleusinatpadaransumberbasispakanlokalterhadapkecernaan, profil VFA rumen danpertumbuhansapi Bali jantanmuda. Prosiding. Seminar Nasional 1. LaboretoriumRisetTerpadu, UNDANA, Kupang
11. Hoover, W.H. and T.K. Miller. 1992. Rumen Digestive Physiology on Microbial Ecology. Aggric. Forestry Exp. Statuon West Virginia, University.
12. Satter, L.D. dan L.L. Slyter. 1974. Effect of amonia concentration on rumen microbial production In vitro. Brit. J. Nutr. 32: 199-208.
13. Hartati E. 1998. SuplementasiMinyakLemurudanSengkedalamRansum yang mengandungSilase Pod kakaodan Urea untukMemacuPertumbuhanSapi Holstein Jantan. Disertasi. Program PascaSarjana, IPB. Bogor.
14. Jenkins, T.C. and D.L. Palmquist. 1984 Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy ations. J. Dairy Sci. 67: 978-986
15. Rastogi, S.C. 1984 Essensial of Animal Physiology. WileyEastem Limited, New Delhi.
16. Schell, T.C. and E.T. Kornegay. 1996 .Zinc concentration in tissue and performance of weaning pigs fed pharmacological levels of zinc from ZnO, Zn-methionine, Zn-lysine or ZnSO: 1584-1593. J. Anim. Sci. 74 (7)"
17. Kinkaid, R.I., R.M. Blauwiekel, dan J.D Cronrath, 1986. Suplementation of cooper sulfat or cooper proteinate for growing calves fed forages containing molybdenum. J. Dairy Sci. 69:160
18. Song, M.K. and N.F. Adham. 1979. Evidence for an important role of prostaglandine E2 and F2 un the regulation of zinc transport in the rat. J. Nutr. 109:2152-2159.
19. Salama Ahmed, A.K, Caja, E. Albanell, X. Such, R. Caslas and J. Plaixats. 2003. Effects of dietary supplements of zinc –methionine on milk production, udder health and zinc metabolism in dairy goats. Journal of Dairy Research. 70: 9-17.
20. Jia, W., X. Zhu, W. Zhang, J. Cheng, C. Gun and Z. Jin. 2009. Effects of source of supplemental zinc on performance, nutrient digestibility and plasma mineral in Cashmere goats. Asian-Austr. J. Anim. Sci. Vol 22, No 12: 1648-1653.
21. Tanuwiria, U.H. 2005. Suplemenseng (Zn) dantembaga (Cu) organikdalamransumberbasislimbahindustriagrountukpemacupertumbuhansapiperahdara. JurnalllmuTernak 5 (1) : 40-46.
22. Belli HLL. The effect of supplementing Bali cattle (Bossondaicus) calves prior weaning on the subsequent reproduction of their dams. Proceeding, Australian Society of Animal Production. Vol. 2B, 2010.
23. Andrea F, Fistini M, Archetti , Emanuele S, Sniffen C, Blag G. Effects of an organic sourse of copper, manganese and zinc on dairy cattle productive performance, health

status and fertility. Abstract. Animal Feed Science and Technology. Published Online, March 02, 2011.