

**Effect of Supplementation of Concentrate containing ZnSO<sub>4</sub> and Zn-Cu isoleucinate on Growth and Post Partum Reproductive Performance of Bali Cows Grazing Native Pastures**

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**ABSTRACT**

**Aims:** This research was carried out to evaluate effect of supplementation of ZnSO<sub>4</sub> 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 twenty seven Bali cows at the last period of pregnancy. The animals were grouped into three treatment groups consisted of grazing on the nearby pasture without supplement (R0), and supplemented with either *Gliricidia* and *Leucaena* leaves (R1), or with concentrate supplement containing 150 mg ZnSO<sub>4</sub> kg<sup>-1</sup> PCF DM and 2% Zn-Cu isoleucinate kg<sup>-1</sup> 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 ZnSO<sub>4</sub>.kg<sup>-1</sup> DM PCF and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> DM diet was very significantly ( $P<0,01$ ) increased the propionate production, NH<sub>3</sub>-N production, plasma glucose, N and energy retention, daily gain of Bali cows in the last period of pregnancy, calf birth weight, estrous cycle and estrous post partum acceleration, and body weight of weaned calves. Average daily gain of Bali cows in the last period of pregnancy, calf birt weight, body weight of weaned calves, estrous cycle and post partum estrous were found on feed supplement treatment containing 150 mg ZnSO<sub>4</sub> kg<sup>-1</sup> DM of PCF and 2% Zn-Cu isoleucinate kg<sup>-1</sup> DM of diet.

**Conclusion:** Overall, the results of this work showed that basal diet supplemented with PCF containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> DM PCF and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> 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.

*Keywords: ZnSO<sub>4</sub>, 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 function and 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 Cu particularly 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<sup>-1</sup> DM and 15 mg.kg<sup>-1</sup> DM respectively [7]. Therefore, providing supplemental feeds containing Zn may improve the performance of grazing cattle in the province.

Several experiments have been conducted on the supplementation of ZnSO<sub>4</sub> 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 ZnSO<sub>4</sub>.kg<sup>-1</sup> DM and 2% Zn-Cu isoleucinate [8]. This positive effect of the supplementation ZnSO<sub>4</sub> 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<sub>4</sub> and Zn-Cu isoleucinate on grazing cows particularly their pre-and post-partum reproductive performance has been largely unknown. Therefore, the objective of this experiment was to investigate the effect of supplementing a concentrate containing ZnSO<sub>4</sub> and Zn-Cu isoleucinate on the performance at the last period of pregnancy and the post-partum reproductive performance of grazing Bali native pasture.

## **2. MATERIAL AND METHODS**

### **2.1 Animals and Experimental Design**

#### 2.1.1. Location and Duration of Experiment

This research was carried out in the Village of Tilong, Kupang Regency, Province of East Nusa Tenggara (ENT), Indonesia. The experiment lasted for six months which consisted of two weeks of adaptation period and ten weeks of data collection.

#### 2.1.2. Animals

As many as 27 Bali cows at the last three-mester of pregnancy were involved in the present experiment. Those animals were owned by a group of farmers who release their cows for grazing in the nearby native pastures together and they have a common barn for their animals.

#### 2.1.3. Experimental Design

The experiment was following a randomized block design with three treatments and 9 replications. The animals 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 (*Gliricida sepium*) and leucaena (*Leucaena leucocephala*) leaves (R1) or a concentrate feed

containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2 % Zn-Cu isoleucinate (3000 ppm Zn and 500 ppm Cu).kg<sup>-1</sup> 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).

Table 1. Chemical composition of the concentrate <sup>\*)</sup>

Feed stuffs (FS)	Composition (%)	PS CP (%)	PS TDN (%)	Concentrate CP (%)	Concentrate TDN (%)
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
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 at 7.00 before the cows were released for grazing. Drinking water was available *ad libitum* on the pasture as well as in the barn during night time.

## 2.2 Variables and Measurements

### 2.2.1. Blood Glucose Concentration

To estimate blood glucose concentration, blood sample was taken via jugular 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.

### 2.2.2. Rumen Amonia-N and VFA Concentration

Rumen fluid was collected through oropharyngeal tube connected to a vacuum pump at similar day as the blood sampling. 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 NH<sub>3</sub> and individual VFA concentration. Assessment of Amonia-N (NH<sub>3</sub>) 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.

### 2.2.3. Body Weight Gain of Cows during Pregnancy and Post-partum

Growth at the last period of pregnant i.e. daily cows body weight gain and fetus growth, and calf birth weight were recorded in the present experiment. Weighing of experimental animal was conducted once every two weeks during pregnancy and post-partum.

#### 2.2.4. Post-partum Reproductive Efficiency

The post-partum reproductive efficiency was determined based on the length of estrous cycles, post-partum unestrous interval as well as by weaning weight of the calves. Cows were monitored twice a day, ie. in the morning before grazing and in the afternoon after returning from grazing. Calves were weighed once in two week in the morning before released for grazing.

### 2.2. Intake, Digestibility and Nitrogen Retention

Nutrients intake and digestibility were estimated based on the method described by Ranjhan [9]. Chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) technique was used to estimate the intake and digestibility of dry matter. After 24 hours of Cr<sub>2</sub>O<sub>3</sub> administration to cows, fecal samples were collected and separated within 24 hours. Total fecal samples were also used to analyze the nutrient contents in the feces. The formulas used were as followed:

$$\text{Dry matter of feces (g/day)} = \frac{\sum \text{Cr}_2\text{O}_3 \text{ intake daily}}{\text{g Cr}_2\text{O}_3 \text{ per g of feces}}$$

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

$$\text{Dry matter intake (kg/hour)} = \frac{\text{fecal output of dry matter}}{\text{Indigestibility}} \times 100$$

$$\text{Zn absorption (mg. day}^{-1}\text{)} = \text{Zn consumption (mg)} - \text{Zn in feces (mg)}$$

$$\text{Cu absorption (mg. day}^{-1}\text{)} = \text{Cu consumption (mg)} - \text{Cu in feces (mg)}$$

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.

### 2.3 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 NH<sub>3</sub>-N 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 ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu

isoleucinate. kg<sup>-1</sup> of ration DM but not with the supplementation of Gliricidia and Leucaena leaves.

Table 2. The average of pH, NH<sub>3</sub>-N and VFA partial concentration in rumen fluid of Bali cows grazing native pasture supplemented with legumes or concentrate containing ZnSO<sub>4</sub>.kg-1 CFP DM and 2% Zn-Cu isoleucinate

Item	Treatment		
	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
pH	6.61± 0.72 <sup>a</sup>	6.75 ± 0.19 <sup>ab</sup>	6.80± 0.64 <sup>b</sup>
NH <sub>3</sub> -N (mM)	3,54±0,71 <sup>a</sup>	4,59±0,01 <sup>a</sup>	6,76±1,11 <sup>b</sup>
VFA:			
Acetate (C2) (mM)	16.46±6.49 <sup>a</sup>	7.23±2.01 <sup>b</sup>	6.53±2.03 <sup>b</sup>
Propionate (C3) (mM)	2.94±1.71 <sup>a</sup>	3.29±1.05 <sup>a</sup>	7.95±1.12 <sup>b</sup>
Iso butyrate (i C4) (mM)	0.73±0.10 <sup>a</sup>	0.52±0.11 <sup>b</sup>	0.18±0.04 <sup>c</sup>
n Butyrate (n butyrate) (mM)	6.39±2.46 <sup>a</sup>	3.38±1.75 <sup>b</sup>	0.70±0.11 <sup>c</sup>

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

Result of the present experiment showed that there was a highly significant difference (P<0.01) in the rumen pH change between Bali cows fed with CFP containing 150 mg ZnSO<sub>4</sub>.kg-1 CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM and those in the control group. 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 ZnSO<sub>4</sub>.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 fermentation inside the rumen [11].

Supplying the raised semi-intensive Bali cows at the last pregnancy period with CFP containing coconut oil 1.5% diet DM (Table 1) and 150 mg ZnSO<sub>4</sub>.kg-1 of CFP DM and 2 % Zn-Cu isoleucinate. kg-1 of DM ration resulted in significant difference of the increasing N-NH<sub>3</sub> in the rumen. This may as a result of increasing of the microbial protein synthesis and decreasing microbial lyses 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 in the cows that were fed only with CFP containing ZnSO<sub>4</sub> 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 ZnSO<sub>4</sub>.kg-1 of CFP DM and 2% Zn-Cu isoleucinate. kg-1 of ration DM. The CFP supplementation containing 150 mg ZnSO<sub>4</sub>.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 NH<sub>3</sub>-N/L in rumen fluid that is required for maximum micro-organisms' growth. The average of NH<sub>3</sub>-N found in this study was around 6.7 mM NH<sub>3</sub>-N/L. The higher content of NH<sub>3</sub>-N 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 ZnSO<sub>4</sub>.kg<sup>-1</sup> of CFP DM and 2 % Zn-Cu isoleucinate resulted in highly significant difference on individual 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, iso and n-butyrate) profile, and concentration of acetate and propionate in rumen fluid by supplying CFP which was supplemented with 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> 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 bioavailability and biological value of micro mineral Zn in Zn-organic is higher than Zn inorganic [16]. It is also found that the biological value and bioavailability of Cu in Cu-proteinates is higher than in CuSO<sub>4</sub> [17]. Kincaid et al. [17] and Schell and Kornegay [16] showed that Zn-Cu proteinates and Zn-Cu isoleucinate can not be fermented by rumen micro-organism, but it can be hydrolyzed by pepsin. In other word, the availability Zn-Cu proteinates or Zn-Cu isoleucinate in pasca rumen show that increased plasma Cu concentration.

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 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> of CFP DM and 2% Zn-Cu isoleucinate kg<sup>-1</sup> 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, there is a highly significant increase of body weight gain and fetus growth at the last period of pregnancy in supplemented Bali cows, and the highest increase was recorded in cows that were supplemented with 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> of ration DM in CFP. Improving body weight gain of Bali cows and fetus growth in this treatment may be contributed by the increase of Zn and Cu absorption (Tabel 3), This study was consistent with the result in young male Bali cattle as reported by Hartati et al., [8], The increased Zn absorption may result in improvement of various enzymes activities especially alkaline phosphatase. The availability of glucose as energy source for fetus growth is significantly higher of cows fed CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> of ration DM than the unsupplemented Bali cows during the last trimester of pregnancy period. It is useful for increasing the supply of metabolizable nutrients which 80% of them are responsible for fetus development and growth as well as improving peptidase activity in both protein and amino acid metabolism.

This in turn results in improved birth weight of Bali calves in the supplemented Bali cows (Table 3)

Beside the above profits, 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 energy metabolism. Beside increasing the activity of alkaline phosphatase, the supplementation of ZnSO<sub>4</sub> 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 as reported by Hartati et al. [8].

Table 3. Mean ( ± SD) 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 <sup>-1</sup> )	142,3± 0,21 <sup>a</sup>	289,5±0,019 <sup>b</sup>	472,3±0,06 <sup>c</sup>
Calf Birth weight (kg)	11,13±1,17 <sup>a</sup>	12,14±0,85 <sup>a</sup>	14,19±1,00 <sup>b</sup>
Blood glucose concentration (mg.dL <sup>-1</sup> )	90,50± 0,72 <sup>a</sup>	92,91±1,56 <sup>b</sup>	94,61± 0,64 <sup>c</sup>
Zn absorption (ppm)	40.28 ± 4.68 <sup>a</sup>	47.31± 2.71 <sup>b</sup>	77.19 ± 1.39 <sup>c</sup>
Cu absorption (ppm)	26.79 ± 3.01 <sup>a</sup>	30.19 ± 1.96 <sup>b</sup>	38.93 ± 2.19 <sup>c</sup>

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 ZnSO<sub>4</sub>.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 % 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 ZnSO<sub>4</sub> 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 ZnSO<sub>4</sub> kg-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 ZnSO<sub>4</sub> 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<sup>-1</sup> DM either as ZnSO<sub>4</sub> or Zn-Methionin in basal diet containing 23.3 mg Zn.kg<sup>-1</sup> 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 Tanuwiria [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 Bali cows at the last four month of pregnancy period with the supplement containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> of ration DM significantly increased the weaning of weight, estrous cycle and post-partum unestrus interval. The highest increase was recorded in Bali cows supplemented with CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> of CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> of ration DM. The increase of weaning weight is probably due to ability of the cows that were given CFP containing ZnSO<sub>4</sub> and Zn-Cu isoleucinate to sustain longer lactation period compared to the unsupplemented cows as milk provides good enough nutrients 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. Whereas, the availability of Cu tends to increase the role of growth hormone. This is because diet containing 1.5% coconut oil that 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 Bali 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 ZnSO<sub>4</sub> 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 and more effect was recorded with the supplementation of CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> of ration DM than that of gliricidia and leucaena leaves.

Table 4. Mean ( $\pm$  SD) of 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 <sup>-1</sup> )	171,4 $\pm$ 0,02 <sup>a</sup>	266,0 $\pm$ 0,05 <sup>b</sup>	376,2 $\pm$ 0,02 <sup>c</sup>
Weaning weight (kg)	44,98 $\pm$ 2,45 <sup>a</sup>	63,27 $\pm$ 2,17 <sup>b</sup>	85,51 $\pm$ 5,72 <sup>c</sup>

Estrous Cycle (day)	22,5 ± 0,50 <sup>a</sup>	21,0 ± 0,0 <sup>b</sup>	21,0 ± 0,0 <sup>b</sup>
Postpartum Anestrus interval (month)	5,58 ± 0,51 <sup>a</sup>	4,57 ± 0,53 <sup>b</sup>	4,22 ± 0,44 <sup>b</sup>
Blood glucose concentration (mg.dL <sup>-1</sup> )	83,98 ± 0,56 <sup>a</sup>	85,16 ± 0,49 <sup>b</sup>	86,79 ± 0,56 <sup>c</sup>
Blood Hb (mg.dl <sup>-1</sup> )	13,06 ± 0,46 <sup>a</sup>	13,66 ± 0,52 <sup>b</sup>	14,78 ± 0,69 <sup>c</sup>
N retention (g)	7.14 ± 1.59 <sup>a</sup>	33.55 ± 8.12 <sup>b</sup>	59.94 ± 8.74 <sup>c</sup>
Energy retention (kcal)	6.96 ± 0.59 <sup>a</sup>	8.99 ± 1.76 <sup>b</sup>	14.24 ± 1.76 <sup>c</sup>

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 ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate kg<sup>-1</sup> 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 ZnSO<sub>4</sub>.kg<sup>-1</sup> of CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> 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.

Supplementing Bali cows grazing native pastures particularly with concentrate containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> of ration DM significantly improved ( $P < 0.05$ ) postpartum blood glucose levels. 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 cow's blood glucose levels was observed higher in Bali cows fed with CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> CFP DM and 2% Zn-Cu isoleucinate. kg<sup>-1</sup> 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 ZnSO<sub>4</sub> 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 Mn sulphates 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) containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> DM of CFP and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> DM of ration increases the ammonia (NH<sub>3</sub>) concentration and improves propionate concentration which plays role as precursor in increasing plasma glucose, increase the daily body weight gain of Bali cows at the last period of pregnancy increased weaning weight of Bali calves. The supplementation also accelerates estrous cycle from 22.5 days to 21 and shortenes the post partum unestrous interval. Overall, the supplementation CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> DM of CFP and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> DM of ration is able to improve the growth of the last period of pregnancy and the post partum reproductive efficiency of Bali cows grazing native pastures. CFP containing 150 mg ZnSO<sub>4</sub>.kg<sup>-1</sup> DM of CFP and 2% Zn-Cu isoleucinate.kg<sup>-1</sup> DM of ration is the better supplement compared to legume forage, i.e. Glicidia and Leucaena leaves,

#### **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.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

#### **AUTHORS' CONTRIBUTIONS**

Hartati designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Lestari and Kleden managed the analyses of the study. Telupere and Ola managed the literature searches. Jelantik involved in data collection and edited the manuscript. All authors read and approved the final manuscript.

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