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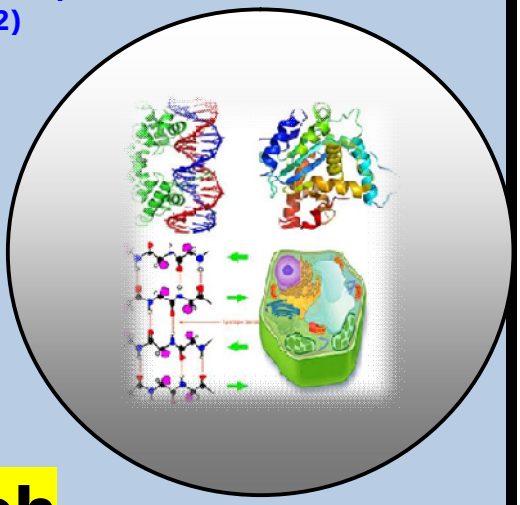

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RESEARCH PAPER

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Optimizing Microbial Protein Synthesis in the Rumen through Supplementation with Vitamin and Mineral in Ration Based on King Grass to increase Bali Cattle Productivity

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ABSTRACT

Bali cattle have a great potency to supply National meet demand which increase progressively every year. The main constrain in Bali cattle farming is the deficiency in trace minerals on native grass resulting in low Bali cattle productivity. The present study was done to determine the effect of vitamin-mineral supplementation in the ration based on king grass on rumen microbial protein synthesis and its relation to productivity of Bali cattle steer. Randomized Complete Block Design was used in this study consisted of four treatments and five groups based on differences in live weight cattle. Treatment consisting of four treatments and five groups based on differences in live weight of cattle. Treatments consist of: S0 = concentrate as much as 5 kg + king grass given ad libitum, S1, S2, and S3 = S0 successively added 0.1%, 0.2% and 0.3% vitamin-mineral in concentrate. Variables observed were intake and ration digestibility, product of rumen fermentation, deposition of nutrients, microbial protein synthesis in the rumen, live weight gain of the animals, and feed efficiencies. The data obtained were analyzed by variance, and regression analysis used to predict the optimal level of supplementation. Results showed that vitamin-mineral supplementation has significantly (P <0.05) effect to most of the observed variables, except the coefficient of rations digestibility. Supplementation levels of 0.2 to 0.3% can reduce the consumption of nutrients, but supplementation levels of 0.1% can increase concentration of propionate acid up to 37%, 12% retention of energy, efficient use of the ration 16%, microbial protein synthesis 15%, reduce methane emissions of up to 18%, and can increase live weight gain of Bali cattle steer up to 14% (0.58 vs. 0.66 kg/day) than cattle without supplements. The research concluded that vitamin-mineral supplementation 0.1 to 0.3% in ration based on king grass can increase microbial protein synthesis in the rumen and live weigh gain of Bali cattle steer, and there is a clear relationship between microbial protein synthesis (X) with live weight gain of cattle (Y) which follows the equation: Y = 0.002X - 0.002, R^2 = 0.73

Keywords: Bali Cattle, Microbial Protein, Supplementation and Vitamin-Mineral.

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INTRODUCTION

Bali cattle are considered as Indonesian native germ plasma that should be preserved and developed optimally in order to improve the welfare of society and the nation of Indonesia. Approximately 55% farmers in most parts of Indonesia raise this breed of cattle because they are easily maintained and have many advantages. Bali cattle breeding has significantly contributed to increasing farmers' income breeders and acceptance (PAD), as well as the beef supply in order to fulfill domestic needs (Direktorat Perbibitan, Dir. Jen Peternakan Deptan and PSP3-IPB, 2005).

Bali cattle belong to ruminants which have rumen. Rumen microbes as biological machines in ruminants have a large role on the productivity of livestock. Rumen is composed of a number of bacteria, protozoa and fungi contained in the ruminant rumen in general and Bali cattle in particular. One of the important roles of rumen microbes are the ability to digest the nutrients contained in food such as carbohydrates, fats, and proteins with enzymes produced. Results from digestion are utilized by beef cattle for growth and production. In addition to exploiting the results of the digestion of nutrients by the animal, cattle can also take advantage of microbial protein for growth. Numerous studies have indicated that rumen microbial protein production correlated positively with the productivity of Bali cattle (Partama et al., 2007a, Partama et al., 2007b). This proves the importance of optimizing bioprocess to improve rumen microbial protein synthesis that significantly affects the growth of cattle. Optimizing rumen microbial protein production in Bali cattle can still be improved to achieve maximum growth of Bali cattle. Ways that are commonly used are to provide nutrient content of diets with adequate and balanced. Adequacy of nutrients such as proteins, carbohydrates, fats, vitamins and minerals is crucial for rumen microbial protein production. However, the results showed that the mineral and vitamin content of animal feed ingredients in the tropics in general and Indonesia in particular is low (Kaunang, 2004). Therefore, mineral and vitamin supplementation in ruminant rations is important in enhancing cattle productivity. Supplementation of vitamins and minerals in the diet has been shown to increase the productivity of Bali cattle (Partama et al., 2003; Partama, 2006). Vitamins and minerals can stimulate productivity of microbial protein synthesis and optimization of the rumen digestive functions of fiber (Wanapati and Sommart, 1992, Partama et al., 2007a; Partama et al., 2007b; Astawa, 2007; Joy, 2008), and high animal productivity is also a reflection of the high microbial protein synthesis (Firkin et al., 2006; Mullik, 2007, Partama et al, 2007a; Partama et al., 2007b). Thus, the success of rumen microbial growth spurred a great effect on the fulfillment of amino acids for ruminants. Mixture of minerals and vitamins not only supplemented to meet the needs of microbes, but also to meet the needs of livestock. In this regard, the research is feasible to determine optimal microbial protein synthesis in relation to live weight increase in fattening Bali cattle fed King grass-based rations with vitamin and mineral supplementation in concentrate.

MATERIAL AND METHODS

This study consisted of a series of field and laboratory experiments. Field trials conducted in the Village Serongga, district of Gianyar, Gianyar regency. Laboratory tests were conducted at the Lab.

Nutrition and Food Livestock, Faculty of Animal Husbandry- Udayana University, and Lab. Analytical Udayana University. Experiments were conducted for six months, starting from the preparation up to the observation or measurement activities in the field.

The experiment was conducted in individual cages. Cage is designed to meet the maintenance requirements of fattening Bali cattle. Twenty (20) individual cages were required to accommodate 20 Bali cattle steers with an average live weight of 319 kg or with the range of 279-367 kg. Cattle were randomly placed in individual cages with a capacity of one larvae per cage and given ration treatment according to the experimental design used.

Ration materials consist of, King grass, concentrates and pignox (commercial product as a source of vitamins and minerals). The materials used to formulate diets with cedar multi vitamin and mineral supplementation differently.

Ration treatments consisted of King grass, concentrates, and pignox (commercial product as a source of vitamins and minerals). The materials were used to formulate diets with cedar multi vitamin and mineral supplementation differently.

Ration treatments consisted of King grass and concentrates supplemented with pignox. There were four treatments in concentrate rations for four treatments, i.e. S0, S1, S2, and S3. Concentrate on S0 is without adding pignox, while concentrate on the S1, S2, and S3 were supplemented with pignox at 0.1%, 0.2% and 0.3%, respectively (Table 1).

Provision of rations conducted two times a day in the morning at 9:00 and lunch at 14.00 pm. Five (5) kg of concentrate was given per cattle per day in two times provision in the morning and afternoon, as mentioned above. King grass was given unlimited (*ad libitum*). The concentrate was given first and followed the King grass on every provision of both morning and afternoon. Similarly, water was supplied *ad libitum* provided separately with the feeding.

Table 1. Nutrient content of the experiment diets.

Nutrient	Concentrates				King grass
	S0	S1	S2	S3	
Dry matter (%)	87,64	87,64	87,64	87,64	24,80
Organic matter(%)	64,13	64,13	64,13	64,13	71,84
Crude protein (%)	12,13	12,13	12,13	12,13	5,01
Crude fiber (%)	7,76	7,76	7,76	7,76	27,20
Energy (GE, Mcal/kg)	3,22	3,22	3,22	3,22	3,39
Sulfur (S, ppm)	685,50	694,09	702,68	711,28	-
Zinc (Zn, ppm)	45,09	65,09	85,09	105,09	26,12

Description: S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate at 0%, 0.1%, 0.2% and 0.3%, respectively.

Experimental Design

This research use randomized block design with four treatments ration S0, S1, S2, and S3 and five groups based on the weight of livestock. Thus there were 20 male Bali cattle in this experiment. One unit was the first experiment in which male Bali cattle were randomly placed in individual cages in accordance with the experimental design. Dietary treatments are: S0 = 5 kg concentrate + King grass was given *ad libitum*; S1 = S0 + 0.1% pignox in concentrate; S2 = S0 + 0.2% pignox in concentrate; and S3 = S0 + 0.3% pignox in concentrate.

Observed Variables

Variables observed were dry matter intake (concentrate and grass) and nutrients, ration digestibility, rumen fluid pH and ammonia, volatile fatty acids (VFA), methane gas emissions and energy, nitrogen retention, deposition of nutrients through the conversion of body composition (Bartle et al., 1983), levels of urinary alllantoin to calculate rumen microbial protein production (Bowen, 2003), cattle weight gain, and feed efficiencies (FCR = feed conversion ratio).

Variables Measurement Procedure

Live Weight Gain. Live weight gain of cattle was obtained by calculating the difference between the initial weight and final weight. Furthermore average daily live gain weight can be detected by dividing the weight difference with the time trial. Livestock weight was measured every two weeks in the morning before the animals were given food and water. Weighing was done by using electronic scales with a capacity of 1000 kg with accuracy 100 g.

Dry Matter and Nutrient Consumption

Ration dry matter consumption was measured by calculating the difference between the amount of ration (weight) given and the remaining rations that there were not consumed. Steaming feed intake is done every day until the experiment ended. To determine the nutrient content of rations, samples of rations were analyzed. Furthermore, the amount of nutrients consumed can be detected by using the equation:

Nutrient consumption = total feed intake x % of dry matter x % of nutrient.

Analysis of protein samples was done with the Macro-Kjedhal method (AOAC, 1980). Ash content determined by burning samples in a furnace at a temperature of 600 ^{0}C for six hours, while determination the energy content of the samples was carried out using adiabatic bomb calorimeter by analyzing 1 g of sample.

Ration digestibility

Digestibility and nutrient retention of the four experimental rations can be detected by recording every day for 7-9 days (total collection period) to the number given rations, *excretion of feces and urine*. Digestibility and nutrient retention coefficient can be calculated using the formula:

C = (K - F) / K X 100% and RN = K - F - U

By understanding: C = coefficient of digestibility (%); RN = retention of nutrients; K = nutrients consumed; F = nutrients removed through feces and U = nutrients removed through urine.

The Acidity level (pH) Rumen Fluid

Acidity is measured using the rumen fluid pH meter Hanna HI 9025 type. Rumen fluid sampling was done three hours after the cattle were fed, using vacuum suction through the mouth as much as 10 ml per cattle. Rumen fluid pH value is known by looking at the quantum of pH meters monitor screen.

Ammonia of Rumen Fluid

Concentrations of N-ammonia (NH_3-N) of rumen fluid were determined by micro-diffusion technique of Conway (Department of Dairy Science, 1966). A total of 1 ml supernatant fluid was placed in one side of the cup near Conway and on the other hand is placed one ml of saturated NaOH solution. The central part was placed a cup Conway ml boric acid solution with indicator. The cup sealed with petroleum jelly assistance. Supernatant and the NaOH solution were mixed evenly and shake the cup. Ammonia released from the reaction between the two materials will then be arrested by the boric acid which is shown by color changes. After 24 hours, ammonium borate was titrated with solution 0.005 N H_2SO_4 until the color changes to the original color of boric acid.

N-ammonia concentration is calculated using the formula:

N-Ammonia = (ml H_2SO_4 X NH_2SO_4 X 1000) mM

Total VFA Concentration

Total VFA concentration analysis was performed using Steam Distillation (Department of Dairy Science, 1966). A total of 5 ml of rumen fluid supernatant sample was introduction into the distillation tube Markham heated with hot water boiled in a flask distillers. Tube was sealed immediately after addition of 1 ml solution of 15% H_2SO_4 solution. VFA will be driven by hot water vapor condensed through cooling tubes and then stored with the previous erlenmayer filled with 0.5 N NaOH up to 100-300 ml. Then 2-3 drops of indicator were added to the next fenolpalin performed titration with 0.5 N HCl. Titration is terminated at the time the starting point of a pink color change became clear. Blank titration was also performed on 5 ml of NaOH. Total VFA concentration was calculated using the following formula:

Total VFA = (bs) X NHCl X 1000 / 5 mM

By understanding:

s = Volume of titrant sample (ml)

b = Volume of titrant blank (ml)

N = Normality of HCl solution

Individual VFA Concentration

Analysis of individual VFA levels was conducted by gas chromatography technique. Rumen fluid was immediately disentrifuged at a speed 10.000 rpm for 15 minutes at a temperature of 4 ºC to obtain supernatants. A total of 2 ml supernatant was taken with a pipette to be inserted into a small plastic tube and then closed. Into these tubes 30 mg 5 sulphosalicylic acid (C_6H_3 (OH) $SO_3 2H_2O$), was added, then whipped and then were centrifuged (3000 rpm for 10 minutes at a temperature of 4 ºC), then filtered with melipori in order to obtain clear liquid. A total of 1 ml of fluid was injected into the gas chromatography; standard solution of VFA was firstly injected.

Concentrations of individual VFA (cM) in rumen fluid samples can be calculated using the following formula:

(cM) = (High Sample/High Standard) x Standard Concentration

Methane gas emissions (M) can be calculated using the formula: M = 0.5 A - 0.25 P + 0.5 B with the understanding M = Methane gas, A = Acetic acid, P = propionate acid, and B = butyric acid. Methane energy value (Mcal) = 0.2108 x mol Methane.

Body Composition

Body composition was measured using a technique of Urea (Urea Space) according to the method of Bartle et al. (1983). This measurement was done once, at the end of the experiment. Measurement procedure was as follows: Blood samples (10 ml) were taken from the jugular vein. Then 20% urea as much as 0.65 cc was injected into the circulation of blood through the jugular vein. After 12 minutes of injection of urea, blood sample was taken again as much as 5 cc. The blood samples were then centrifuged to obtain plasma fluid. Then the plasma was analyzed to determine levels of blood urea by the method of production of Urea Kit Roche, both before and after injection of urea. Body composition can be determined by calculating a urea according to the formula suggested Bartle et al. (1983) as follows:

$$\text{Urea Space}\,(\%) = \frac{\text{urea injected (mg)}}{10\ \text{X live weight X change of blood urea content (mg)}}$$

Empty body water (EBW = empty body water), body fat and body protein (empty body protein) was determined by the following formula:

Water bodies (%) = 59.1 + 0.22 RU - 0.04BH (Rule et al., 1986), where UR = urea space (%) and BH = live weight (kg), body fat (%) = 5.19 - 0.31 RU- 0.05 BH. and body protein (%) = 5.16 + 0.07 RU - 0.001 BH.

Deposition of Nutrients

Deposition of nutrients (fat, protein, and minerals) can be calculated by converting weight daily life with body composition. Based on this nutrient deposition, it can be calculated the energy retention with the provisions of 1g of fat deposition is equivalent to 9.32 kcal, while the deposition of 1 g protein, equivalent to 5.5 kcal (Ørskov and Ryle, 1990). So the retention of energy per day per cattle can be calculated by summing the energy content of the deposition of body fat and protein per cattle per day.

Urine Allantoin Levels

Allantoin concentration in urine was measured by the method of Matsumoto et al. (1995). Microbial protein supply in ruminants can be predicted with the content of purine derivatives in urine. Ruminant animal feed generally contain little purine and most experienced extensive degradation as a result of microbial fermentation. Purine nucleic acids from the degraded mainly derived from the rumen and excreted in the urine as derivatives, namely hipoxanthin, xanthin, uric acid and allantoin. The proportion of these derivatives in ruminant urine allantoin ranged 60-80%, 10-30%, uric acid and xanthin hipoxanthin added as much as 50-10% (Preston, 1995). In this measurement method, allantoin hydrolyzed in alkaline conditions with a temperature of 100 ^{o}C to allantoat acid which was then degraded into urea and glyoxylate acid in acid solution.

Glyoxylate acid will react with phenilhidrasin hidrochlorid to become phenilhidrazon which then will form a colored compound with potassium ferisianida. Then the solution is read at λ 522 nm. Measurement procedure is as follows: 1 ml of urine sample / standard solution / distilled water into a test tube dipipet. Add 5 ml of distilled water and 1 ml of 0.5 M NaOH, then mix the solution by using a vortex. Place the tube in a boiling water bath (PEG boil bath) for 7 minutes. Furthermore, the tube removed and cooled. Into each tube add 1 ml HCl 0.5 M. Then add phenilhidrasin, and then the tube is inserted back into the water bath for 7 minutes. Afterward, the tube is cooled by Icy alcohol bath a few minutes. Then pipette and mixed with 3 ml of concentrated HCl with 1 ml of potassium ferri cyanide and then transferred into a cuvette at room temperature. Read the mixture at 522 nm and concentration of allantoin can be calculated using the following formula:

Allantoin content = Allantoin standard / Allantoin sample x 100 / 5 (mg/1000ml)

Allantoin levels as a purine derivative in urine is used to predict microbial protein production by using the formula International Atomic Energy Agency (1999), namely:

$Y = 0.85\ X + 0.145W^{0.75}$, Y = total allantoin excretion in urine (mmol / day), X = microbial purines absorbed (mmol/day), $0{,}145W^{0.75}$ = contribution of endogenous purines at 0,145 per kg metabolic weight ($W^{0.75}$) from Bali cattle, and the number 0.85 = coefficient of absorption of purines. Furthermore, the value of X is used to predict rumen microbial nitrogen production using the formula:

Microbial N (g/day) = (70X) / (0.83 x 0.116 x 1000), with the understanding purine digestibility = 0.83; X microbial purines absorbed (mmol / day); 70 = N content of purines (mg/mmol), and 0.116 = 11.6: 100 is the ratio of purine : N-total in the rumen. Furthermore, the microbial nitrogen can be converted into microbial protein by multiplying the number 6.25.

Data Analysis

The data obtained were analyzed by variance. When a significant effect on treatment responce variables was observed, the test was continued by orthogonal contrast test at level 5%. Regression analysis was used to determine the optimal mineral-vitamin supplementation to obtain live weight gain the maximum of Bali cattle in accordance with their genetic potential (Steel and Torrie, 1986).

RESULTS AND DISCUSSION

Effect of Vitamin-Mineral Supplementation on Nutrient Consumption

Vitamin-mineral supplementation significantly ($P<0.05$) effected the grass dry matter intake, total dry matter, organic matter, crude protein, crude fiber, minerals and energy consumption. The highest grass dry matter consumption of cattle without supplementation (S0 = control); it was 2.39 kg/day. However, vitamin-mineral supplementation at 0.1% (S1) in concentrate resulted in grass dry matter intake decreased to 2.29 kg/day, but this decrease was not statistically significant ($P> 0.05$). This becomes a real decline in consumption ($P <0.05$) when cattle were given concentrates with vitamin-mineral supplementation at 0.2% (S2) and 0.3% (S3), respectively of 6% and 12% of the control diet (Table 2).

Total dry matter consumption (concentrate + grass) were also influenced significantly by vitamin-mineral supplementation in concentrate. Supplementation of 0.1% showed no significant difference compared with no supplements, but the dry matter consumption decreased significantly at 360-470 g in the vitamin-mineral supplementation from 0.2 to 0.3% (Table 2)

The highest consumption of organic material in cattle fed diets without vitamin-mineral supplementation of 4.45 kg/day, but this consumption was not significantly different ($P>0.05$) compare with cattle supplemented with 0.1%. This organic matter consumption decreased significantly in cattle fed 0.2% vitamin-mineral supplement and 0.3% respectively to 4.21 and 4.12 kg/day (Table 2).

Vitamin-mineral supplementation had significant effect on consumption of protein rations. Protein consumption per day ranges from 7.32 to 7.92 g/kg$W^{0.75}$, equivalent to 528-571 g per cattle per day, when the average weight of 300 kg cattle. Consumption of protein in cattle fed the control diet was not significantly different in protein intake in cattle fed vitamin-mineral supplement 0.1%. Meanwhile, consumption of protein in cattle feed supplementation with 0.2% and 0.3%, significantly lower than the consumption of protein in cattle fed the control diet (Table 2).

In Table 2 also shows that vitamin-mineral supplementation had significant effect on crude fiber intake in Bali cattle fed King grass-based rations. Increased levels of vitamin-mineral supplementation of 0.1% to 2% and 0.3% in concentrates can reduce consumption of crude fiber and minerals. Meanwhile, vitamin-mineral supplementation of 0.1% was not significantly different from control diet.

Table 2. Nutrient consumption in Bali cattle fed King grass-based rations with vitamin mineral supplementation.

Variables	Supplementation Treatment			
	S0	S1	S2	S3
Consumption:				
Dry matter (kg/d):				
• Concentrate	4,26[a]	4,29[a]	4,05[a]	4,08[a]
• Grass	2,39[b]	2,29[b]	2,24[a]	2,10[a]
• Total	6,65[b]	6,58[b]	6,29[a]	6,18[a]
Organic matter (kg/d)	4,45[b]	4,40[b]	4,21[a]	4,12[a]
Crude protein (g/kg$W^{0,75}$/d)	7,92[b]	7,87[b]	7,44[a]	7,32[a]
Crude fiber (g/kg$W^{0,75}$/d)	12,18[b]	11,84[b]	11,39[a]	10,83[a]
Minerals (g/kg$W^{0,75}$/d)	17,35[b]	17,19[b]	16,28[a]	15,96[a]
Energy (Kcal/kg$W^{0,75}$/d)	271,52[b]	267,47[b]	254,54[a]	247,33[a]

Description: Variables with similar superscript number did not significantly different at $P<0.05$ when compared to orthogonal contrast test; S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate at 0%, 0.1%, 0.2% and 0.3%, resvectively. $W^{0,75}$ = metabolic weight of cattle; d = day.

The range of crude fiber intake was 10.83 to 12.18 g/kgW$^{0.75}$/d, while the consumption of minerals ranging from 15.96 to 17.35 g/ kgW$^{0.75}$/d. Consumption of crude fiber and minerals are the highest in cattle fed the control diet and lowest in cattle fed vitamin-mineral supplement 0.3% in concentrate.

Energy consumption was also affected by vitamin-mineral supplementation in cattle fed basic ration king grass. Ration energy consumption in cattle fed the highest vitamin-mineral supplementation of 0.1% in concentrate, but not significantly different from cattle fed a control diet (Table 2). The range of energy consumption is 247.33 to 271.52 Kcal/kgW$^{0.75}$/d, equivalent to 17.8 to 19.6 Mcal per cattle per day, when the average weight of cattle is 300 kg.

Effect of Vitamin-Mineral Supplementation on Nutrient Digestibility

Vitamin-mineral supplementation did not significantly affect digestibility coefficient of dry matter, organic matter, crude protein, crude fiber, and energy, but significant effect on nitrogen retention (Table 3). Dry matter digestibility coefficient ranged 69-71%, the highest was in cattle fed the control diet and lowest in cattle fed vitamin-mineral supplements in concentrate 0.2%, but all digestibility coefficients showed no significant difference (P> 0.05).

Ration digestibility coefficient of organic matter ranging from 68-70%, the highest digestibility coefficient was also for cattle fed the control diet and lowest at 0.2% supplementation treatment. Digestibility coefficients of the five groups of cattle that were given four treatments of vitamin-mineral supplementation in concentrate were statistically significantly differed.

Vitamin-mineral supplementation resulted in decreased digestibility coefficient of crude protein and crude fiber in Bali cattle fed King grass based rations, but this decrease was not statistically significant. Crude protein digestibility coefficients ranged 71-73%, while crude fiber digestibility coefficient ranges of 41-48%. The coefficient of digestibility was highest in cattle fed the control diet (Table 3).

In Table 3, it was indicated that little energy digestibility coefficient increased in cattle fed vitamin-mineral supplementation of 0.1% and 0.3% in concentrate compared with cattle without supplementation, but this increase was not statistically significant (P> 0.05). Energy digestibility coefficient was the highest in cattle with vitamin-mineral supplementation of 0.1% and lowest in cattle fed the control diet and cattle by supplementing 0.2%, are 71% each.

Vitamin-mineral supplementation significantly affected the retention of nitrogen (N) in cattle fed King grass-based rations (Table 3). Vitamin-mineral supplementation at 0.1% in concentrate has not resulted in decreased N retention than cattle without supplementation, but supplementation increased to 0.2% and 0.3% resulted in a decrease in nitrogen retention to 22% and 25%, respectively compared to cattle without supplementation. Overall, N retention ranged from .51 to .68 g/kgW^{0,75}/d, equivalent to 37-49 g per cattle per day when the average live weight of cattle is 300 kg.

Table 3. Nutrient digestibility coefficients and nitrogen retention in Bali cattle fed King grass-based rations with vitamin-mineral supplementation.

Variables	Supplementation treatment			
	S0	S1	S2	S3
Digestibility coefficient :				
• Dry matter (%)	71[a]	70[a]	69[a]	70[a]
• Organic matter (%)	70[a]	69[a]	68[a]	69[a]
• Crude protein (%)	73[a]	72[a]	71[a]	71[a]
• Crude fiber (%)	48[a]	41[a]	41[a]	44[a]
• Energy (%)	71[a]	73[a]	71[a]	72[a]
Nitrogen retention (g/kg$W^{0,75}$/d)	0,68[b]	0,68[b]	0,53[a]	0,51[a]

Description: Variables with similar superscript number did not significantly differ at $P<0.05$ when compared with orthogonal contrast test; S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate at 0%, 0.1%, 0.2% and 0.3%, respectively. $W^{0,75}$ = metabolic weight of cattle; d = day.

Effect of Vitamin-Mineral Supplementation on Rumen Fermentation

Results of fermentation and microbial protein synthesis in Bali cattle fed King grass-based rations were influenced significantly ($P<0.05$) by vitamin-mineral supplementation in concentrate (Table 4). Vitamin-mineral supplementation can reduce cattle rumen pH up to 12% at the level of supplementation of 0.3% when compared with cattle without supplementation. Rumen pH values in this study ranged from 6.0 to 6.8 and the highest pH values in cattle fed diets without vitamin-mineral supplementation, and the pH value was significantly higher than supplementation treatment.

Concentrations of N-NH_3 cattle rumen fluid was also significantly influenced by vitamin-mineral supplementation. Vitamin-mineral supplementation at 0.1% in concentrate produced NH_3-N concentration of rumen fluid that is 15.50 mM highest compared with cattle fed diets without supplements and cattle with vitamin-mineral supplementation of 0.2% and 0.3% in concentrate .

Vitamin-mineral supplementation significantly affected the concentration of volatile fatty acids (VFA) total, acetate, propionate and butyrate in Bali cattle fed King grass-based rations. Total VFA concentration ranged from 155.01 to 198.06 mM and the highest concentration in cattle with vitamin-mineral supplementation 0.3% and the lowest concentration in cattle supplemented with 0.2% level.

Vitamin-mineral supplementation can reduce the concentration of acetic acid significantly up to 10% in cattle with supplementation of 0.1% compared with cattle without supplementation (77.78 mM vs. 86.65 mM). However, improvements in the 0.3% level of supplementation resulted in increased concentration of acetic acid to 113.11 mM, which is the highest concentration, and significantly higher than other treatments. Overall, the concentration of acetic acid ranged from 77.78 to 113.11 mM and the lowest concentrations in cattle with supplementation of 0.1%, but insignificantly different from 0.2% supplementation.

Vitamin-mineral supplementation at 0.1% in concentrate may increase the concentration of cattle rumen fluid propionate acid up to 37% compared with cattle without supplementation (64.21 mM vs. 46.88 mM). However, supplementation increased to 0.3% caused a decrease in propionate acid concentration to 36.38 mM (Table 4).
Butyric acid concentrations have not significantly change when given a vitamin-mineral supplementation up to 0.2% in concentrate. However, increasing levels of supplementation up to 0.3%, causing the concentration of butyric acid increased to 33.28 mM of cattle without supplementation of the acid content butyrate only 21.91 mM (Table 4).

Table 4. Results fermentation and microbial protein synthesis in the rumen of Bali cattle fed King grass-based rations with vitamin-mineral supplementation.

Variables	Supplementation treatment			
	S0	S1	S2	S3
Ruminal pH	$6,8^{c}$	$6,4^{b}$	$6,3^{b}$	$6,0^{a}$
Ruminal N-NH_3 (mM)	$12,30^{a}$	$15,50^{b}$	$12,74^{a}$	$11,92^{a}$
Total ruminal VFA (mM)	$166,33^{a}$	$181,75^{b}$	$155,01^{a}$	$198,06^{b}$
Acetate (mM)	$86,65^{b}$	$77,78^{a}$	$79,27^{a}$	$113,11^{c}$
Propionate (mM)	$46,88^{a}$	$64,21^{b}$	$45,18^{a}$	$36,38^{a}$
Butyrate (mM)	$21,91^{a}$	$24,30^{a}$	$22,14^{a}$	$33,28^{b}$
Methane (mM)	$42,56^{b}$	$34,99^{a}$	$39,41^{a}$	$64,10^{c}$
Microbial Protein Synthesis (MPS, g/d)	$202,24^{a}$	$232,24^{c}$	$225,67^{b}$	$221,46^{b}$
Efficiency of rumen MPS (g/kgOMdR)	110^{a}	126^{b}	133^{b}	126^{b}

Description: Variables with different superscript number differed significantly at $P<0.05$ when compared with orthogonal contrast test; VFA = Volatile Fatty Acids; OMdR = organic matter degraded in the rumen = 0.65 x insoluble organic matter; S0, S1 , S2 and S3 = vitamin-mineral supplementation in concentrate at 0%, 0.1%, 0.2% and 0.3%, respectively.

The results of this study showed that vitamin-mineral supplementation of 0.1 to 0.2% in concentrate to reduce methane emissions at the Bali cattle fed King grass-based rations (Table 4). Vitamin-mineral supplementation at 0.1% in concentrate to reduce methane emissions up to 18% compared with cattle without supplementation (34.99 mM vs. 42.56 mM). However, increasing levels of supplementation up to 0.3% of methane emissions increased by up to 51% of cattle without supplementation (64.10 mM vs. 42.56 mM).
Vitamin-mineral supplementation of 0.1-0.3% in concentrate can significantly increase microbial protein synthesis in Bali cattle fed King grass-based rations (Table 4). Increased microbial protein synthesis was reached 15% in cattle with vitamin-mineral supplementation of 0.1% when compared with cattle without supplementation (232.24 g/day vs 202.24 g/day). Efficiency of rumen microbial protein synthesis, also increased in cattle fed vitamin-mineral supplements. Efficiency of rumen microbial protein synthesis was increased to 21% in cattle with supplementation of 0.2% in concentrate compared with cattle without supplementation (133 vs. 110 g/kg of organic material degraded in the rumen).

Regression analysis shows that there is a real relationship between the level of vitamin-mineral supplementation with rumen microbial protein synthesis of Bali cattle which follows the equation: $Y = 204.1 + 307.7X - 855.4X^2$, with a coefficient of determination (R^2) = 0.501*, with the understanding of X = level of vitamin-mineral supplementation in percent (%), and Y = microbial protein synthesis in g/day (Figure 1).

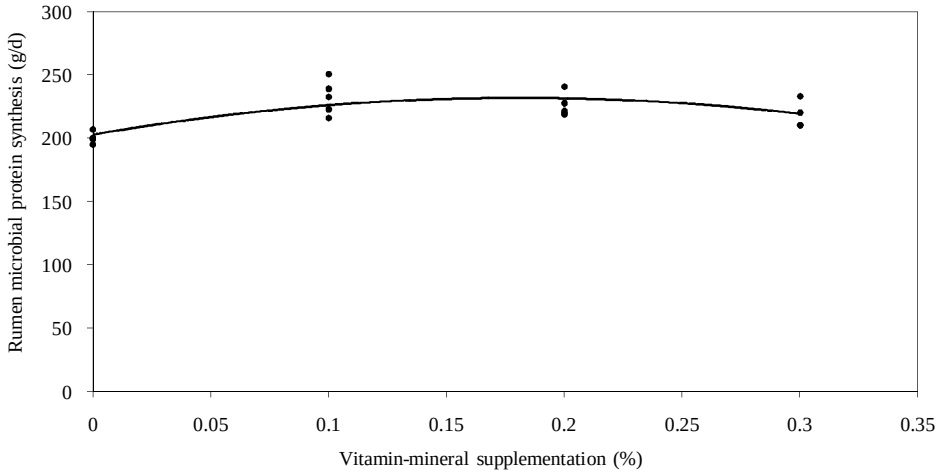

Figure 1. The relationship between vitamin-mineral supplementation with rumen microbial protein synthesis of Bali cattle fed King grass-based rations.

Based on these quadratic equations can be predicted vitamin-mineral supplementation in concentrate optimum is 0.18% which will cause maximum rumen microbial protein synthesis amounted to 231.77 g per day.

Table 5. Deposition of nutrients and energy for productivity of Bali cattle fed King grass-based rations with vitamin-mineral supplementation.

Variables	Supplementation Treatment			
	S0	S1	S2	S3
Deposition of Nutrients:				
• Protein (g/kgW^{0,75}/d)	1,18^a	1,31^b	1,30^b	1,19^a
• Fat (g/kgW^{0,75}/d)	2,6^a	3,0^b	2,9^b	2,7^a
Methane energy (Kcal/kgW^{0,75}/d)	15,45^b	12,46^a	14,07^a	22,71^c
Retention of Energy (NEp, Kcal/kgW^{0,75}/d)	31,14^a	34,92^b	34,64^b	32,06^a
Feed Conversion Ratio (FCR)	11,48^b	10,06^a	9,68^a	10,18^a
Live weight gain (kg/d)	0,58^a	0,66^b	0,65^a	0,61^a

Description: Variables with different superscript number significantly differed at P<0.05 when compared with orthogonal contrast test; NEp = Net energy for production (live weight gain of cattle); FCR = total dry matter intake divided by live weight gain per day of Bali cattle; S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate at 0%, 0.1%, 0.2% and 0.3%, respectively; $W^{0,75}$ = metabolic weight of cattle; d = day.

Effect of Vitamin-Mineral Supplementation on Energy Utilization

Vitamin-mineral supplementation in concentrate significantly affect nutrient deposition, methane energy, energy retention, feed conversion ratio (FCR), and live weight gain of Bali cattle fed King grass-based rations (Table 5). Protein and fat deposition increased significantly due to vitamin-mineral supplementation from 0.1 to 0.2% in concentrate.

Protein deposition ranged from 1.18 to 1.31 g/kgW$^{0.75}$/day equivalent to 85.06 to 94.43 g/head/day, and the highest deposition in cattle with vitamin-mineral supplementation of 0.1% in concentrate. Meanwhile, the deposition of fat ranged from 2.6 to 3.0 g/kgW$^{0.75}$/day equivalent to 187.42 to 216.25 g/head/day when the average live weight of 300 kg of Bali cattle.

Vitamin-mineral supplementation in concentrate has significantly affected the energy lost in the form of methane gas. Increased levels of supplementation up to 0.2% can reduce the energy of methane, but supplementation of 0.3% can increase the energy of methane (Table 5). The energy lost in the form of methane ranged from 12.46 to 22.71 Kcal/kgW$^{0.75}$/day, equivalent to 0.9-1.64 Mcal/head/day when the average weight of 300 kg of Bali cattle.

Energy retention to live weight gain of cattle was also influenced significantly by vitamin-mineral supplementation. Vitamin-mineral supplementation up to 0.2% in concentrate level can increase the energy retention significantly ($P<0.05$) in Bali cattle fed King grass-based rations (Table 5). Supplementation at 0.3% level in concentrate can also increase the retention of energy, but the increase was not statistically significant. Energy retention to added weight of live cattle in this study ranged from 31.14 to 34.92 Kcal/kgW$^{0.75}$/day and equivalent to 2.42 to 2.52 Mcal /head/day, when the average weight of 300 kg. The highest energy retention in cattle with 0.1% supplementation in concentrate and lowest was in cattle without supplementation.

Ration utilization efficiency was also affected by vitamin-mineral supplementation. Vitamin-mineral supplementation can improve the efficiency of feed utilization in Bali cattle characterized by increasingly low value of FCR (feed conversion ratio). In Table 5 it showed the lowest FCR values in cattle with supplementation of 0.2% and the highest was in cattle without supplementation.

Vitamin-mineral supplementation in concentrate may increase the live weight gain of Bali cattle fed King grass-based rations, but supplementation of 0.2 to 0.3% levels did not differ significantly with live weight gain of Bali cattle fed a control diet (Table 5). Added cattle live weight was reached at 0.1% supplementation and the lowest on the control diet. There is a clear relationship between vitamin-mineral supplementation with live weight gain of Bali cattle as following regression quadratic equation: $Y = 0.583 + 0.959\ X - 2.95\ X^2$ with a coefficient of determination (R^2) = 0.414* with the understanding of X = supplementation of vitamin-mineral (%), Y = live weight gain of Bali cattle (kg/day) as seen in Figure 1. From this regression equation can be predicted optimal vitamin-mineral supplementation of 0.16% which produces the maximum live weight gain of Bali cattle 0.66 kg/day.

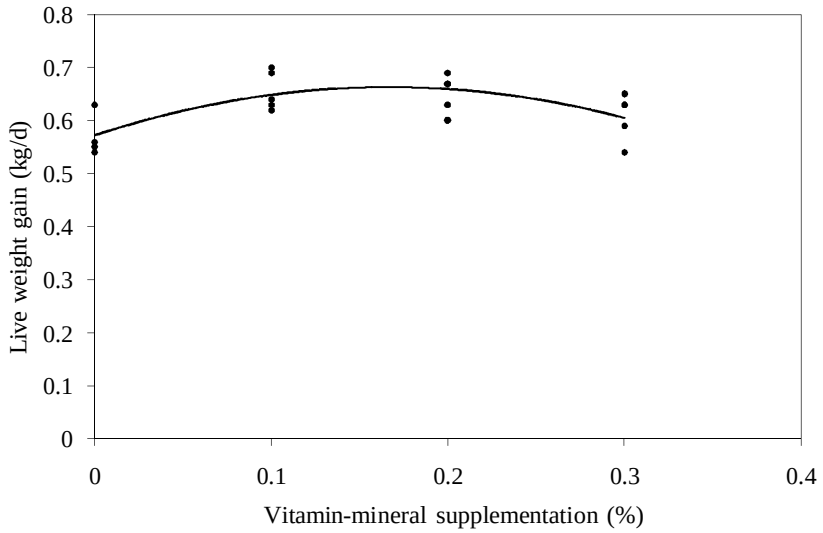

Figure 2. The relationship between vitamin-mineral supplementation with live weight gain of Bali cattle fed King grass-based rations.

DISCUSSION

In principle, feed intake in animals is to meet the nutrient and energy needs. Livestock will stop eating when energy needs are met or stomach has been filled by the feed nutrient needs exceed the capacity, although not yet fulfilled. Level of feed intake is influenced by physiological status of livestock, feed quality and palatability. To obtain the optimal level of consumption requires ration formulations that match the needs of livestock rations containing adequate and balanced nutrient.

Ration is attempted in this study meets the nutrient needs of ration ingredients consisting of 5 kg of concentrate, King grass was given *ad libitum* or given an average of 15 kg per cattle per day, so that these rations containing dried material (BK) 40.51%, crude protein (CP) 10.31%, and energy (GE) 3.27 Mcal/kg, equivalent to 56.29% levels of TDN (Total Digestible Nutrients). However, the results of this experiment showed that feed intake and nutrient levels such as dry matter, organic matter, crude protein, and energy decreased when given vitamin-mineral supplementation in concentrate; especially the level of supplementation of 0.2 to 0.3% caused a decrease in consumption statistically significant (Table 2). This decline in consumption caused by an imbalance of nutrients in diet, especially minerals. This causes the excess mineral supplementation, especially Zn in cattle's digestive tract causes metabolic disturbances, loss of appetite, reduce the accumulation of Fe and Cu in the liver and increase spending on S in the feces (Geogievskii, 1982). Thus, this will suppress metabolism disorders feed intake and nutrients. Decrease in consumption level has not yet led to nutrient-deficient cattle and this can be proved by the accretion of a cattle live weight during the experiment, although feed intake decreased with increasing levels of vitamin-mineral supplementation (Table 2 and Table 5).

Ration digestibility showed no significant difference although feed intake was significantly affected by vitamin-mineral supplementation in concentrate (Table 3). However, there is a trend of increased consumption is followed by an increase in digestibility coefficients. This can be understood that the higher value of digestibility fasten to empty of the digestive tract so that fasten the filling through the higher consumption.

N retention was significantly affected by vitamin-mineral supplementation in concentrate on Bali cattle fed King grass-based rations. Level of supplementation 0.1% to produce N retention the same with ration without supplementation, but N retention decreased when the level of supplementation was increased to 0.2-0.3%. This proves that the plant feed or ruminant feed material is still lacking minerals Zn, which is the level of supplementation of 0.1% would still give positive effect to decrease nitrogen retention when the level of supplementation increased. This represents an indicator of the optimal level of vitamin-mineral supplementation in concentrate. The results of this study support the report Kaunang (2004) that tropical forage plants and especially in Indonesia is truly deficient of minerals, especially Zn.

Vitamin-mineral supplementation significantly affect the results of fermentation and microbial protein synthesis in cattle fed basic Balinese king grass (Table 4). Values of pH decreased with increasing levels of vitamin-mineral supplementation in concentrate, but rumen fluid pH values still within the normal range is 6.0 to 6.8. This condition supports the normal fermentation process took place in the rumen. This can be proved by $N\text{-}NH_3$ concentrations sufficient for microbial protein synthesis. Rumen $N\text{-}NH_3$ concentration in this study ranged from 11.92 to 11.50 mM. The results of this study is higher than reported by Satter and Slyter (1974) cited Sutardi (1997) that the minimum concentration of $N\text{-}NH_3$ is 4 mM and the highest concentration reported by Preston and Leng (1987) cited Sutardi (1997), namely 14 mM. This can be understood that this study used the Bali cattle, while another study did not use Bali cattle, however, $N\text{-}NH_3$ concentration range was still within normal range. This can be proved by the result of fermentation of volatile fatty acids (VFA), which is quite high.

$N\text{-}NH_3$ concentration sufficient to offset the relatively high concentration of VFA (from 166.33 to 198.06 mM) would strongly support the efficiency of rumen microbial protein synthesis. $N\text{-}NH_3$ is the result of protein degradation, while the VFA degradation products from carbohydrates in the rumen. The balance between these two components is an ideal prerequisite for the optimization of microbial protein synthesis. Stern et al. (2006) stated that the rumen bacteria can use protein and carbohydrates as energy sources. Carbohydrate is the main energy source for bacteria, and can also be used as a carbon skeleton that combines with ammonia (NH_3) to rumen microbial protein synthesis.

Vitamin-mineral supplementation of 0.1% in concentrate to give the best effect in the process of fermentation and microbial protein synthesis in cattle fed basic ration of king grass (Table 4). The highest concentration of $N\text{-}NH_3$ followed by the highest propionate acid and the lowest concentration of methane gas caused the highest rumen microbial protein synthesis compared to other treatments. This shows that supplementation at the level of 0.1% produce enough rations balanced nutrients and minerals, especially balance.

Mineral content of Zn to 65.09 ppm (Table 3) in concentrate, slightly higher than the recommendation of Georgievskii (1982), i.e. 40-60 ppm is based on the needs of the cattle outside Indonesia. This is true that mineral nutrient requirements especially of Zn in Bali cattle are higher than the cattle in foreign countries. Zn minerals needed by cattle small amounts (micro minerals), but it has a very important function in the various activities of enzymes in the body of cattle.

There is a clear relationship between levels of vitamin-mineral supplementation with rumen microbial protein synthesis following regression equation: $Y = 204.1 + 307.7\ X - 855.4\ X^2$, with a coefficient of determination (R^2) = 0.501*, with the understanding of X = level of supplementation vitamin-mineral in percent (%), and Y = microbial protein synthesis in g/day. (Figure 1). Regression equations indicate that the levels of vitamin-mineral supplementation at 0.18% couse maximal microbial protein synthesis amounted to 231.77 g per day. This means that supplementation levels above 0.18% has revealed an imbalance of nutrients that can reduce rumen microbial protein synthesis, rumen microbial protein which has a very high biological value for growth or production of beef cattle.

Vitamin-mineral supplementation from 0.1 to 0.2% in concentrate to give a positive influence on the deposition of nutrients (protein and fat), energy utilization and productivity of Bali cattle fed King grass-based ration (Table 5). Increased nutrient deposition was related to the outcome of fermentation in the rumen (Table 4). The high concentration of $N\text{-}NH_3$ and propionate acid and low methane production at the level of supplementation from 0.1 to 0.2% produce microbial protein which synthesis high also. Rumen microbial protein as a major sources of amino acids for the host animal, so the higher rumen microbial protein production of higher protein deposition in the body of cattle. Similarly, the higher is the acid production of the propionate, the higher the nutrient deposition in the form of body fat. Meanwhile, the lower the methane gas production means less energy is wasted so that more energy is stored in the form of animal protein and fat body.

The high methane production in cattle without supplementation and cattle with vitamin-mineral supplementation of 0.3% in line with crude fiber digestibility coefficients (Table 3 and Table 4). The higher is the crude fiber digestibility coefficients are in line with the higher production of methane gas. These data indicate that the pattern of fermentation leads to the portion of acetic acid of a larger line with the increased production of methane gas because crude fiber is structural carbohydrate which the result of fermentation more acetic acid (Arora, 1995). The productions of methane in cattle with high levels of supplementation of 0.3% probably due to the existence of advanced fermentation process so many of liberate H^2 utilized by methane-forming microbes such as *Methanobacterium ruminantium*, and *Methanobacterium mobilis* (Arora, 1995).

Deposition of nutrients (protein and fat) is associated with retention of energy for livestock production. The results of this study show that increasing nutrient deposition higher energy retention to live weight gain of cattle that the higher also (Table 5). This is understandable because the fat and protein the body can be converted into energy which the energy content of one gram of fat and one gram of protein, respectively 9.32 and 5.5 kcal/g (Orskov and Ryle, 1990).

The high nutrient deposition, energy retention and live weight gain in cattle with vitamin-mineral supplementation of 0.1% due to the sufficient and balanced nutrients in the ration. Concentrate with a vitamin-mineral supplementation 0.1% containing ratio of N : S are balanced, and contains enough minerals Zn and S (Table 3). Sulfur is an essential mineral in amino acids synthesis contains sulfur, and is needed in large numbers to microbial protein synthesis. Meanwhile, Zn minerals involve in metalo enzyme synthesis such as DNA and RNA polymerase, alkaline phosphatase, amylase and neutral protease (Jouany, 1991). Thus, this strongly supports rumen microbial protein synthesis and its activity so that the feed material entering the rumen degraded to produce energy efficiently utilized with a low indicator of wasted energy in the form of methane gas.

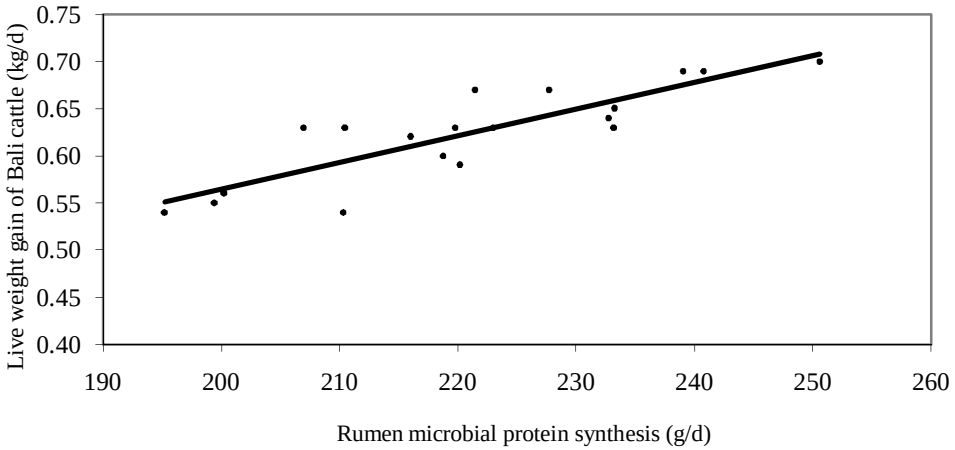

Figure 3. Relationships between rumen microbial protein synthesis with live weight gain of Bali cattle fed King grass-based rations.

The results showed that the apparent link between rumen microbial protein synthesis with live weight gain of Bali cattle fed King grass-based rations with vitamin-mineral supplementation. Relationship following the regression equation: $Y = 0.002X – 0.002$, with coefficients of determination (R^2) = 0.73*, with the understanding of X = microbial protein synthesis (g/day) and Y = live weight gain of cattle (kg/day) (Figure 3). This regression equation means that any increase in rumen microbial protein synthesis for 1 g will be followed by a live weight gain of cattle of 2 g per day. The results of this study support previous research that reported results Partama et al. (2007) that there is a real relationship between microbial protein synthesis with live weight gain of Bali cattle fed rice straw urea ammoniated-based complete rations with supplementation of vitamin-mineral complex.

CONCLUSIONS

The results of this study can be summarized as follows:

Vitamin-mineral supplementation by 0.1-0.3% in concentrate may increase the efficiency of utilization of feed, rumen microbial protein synthesis, deposition of protein and fat, the retention energy, reduce emissions of methane up to 18% at the level of supplementation of 0.1% and can increase live weight gain in Bali cattle fed King grass-based rations.

Retrieved optimum levels of vitamin-mineral supplementation in concentrate is 0.16% which can produce maximum of microbial protein synthesis and live weight gain of Bali cattle.

There is a real relationship between rumen microbial protein synthesis (X) and live weight gain of Bali cattle (Y) fed King grass-based rations with vitamin-mineral supplementation according to the regression equation: $Y = 0.002X - 0.002$, $R^2 = 0.73^*$ which means that any increase in rumen microbial protein synthesis for 1 g will be followed by a live weight gain of Bali cattle of 2 g per day.

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