

Nitrogen balance, microbial protein synthesis and blood metabolites in fattening of male Bali cattle fed ration with different protein levels in smallholder farms

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ABSTRAK

Penelitian bertujuan mengetahui keseimbangan nitrogen, sintesis protein mikrobia, serta metabolit darah dari Sapi Bali jantan yang memperoleh ransum dengan level protein pakan berbeda di peternakan rakyat, Timor Tengah Utara, Nusa Tenggara Timur, Indonesia. Ternak yang digunakan sebanyak 18 ekor dengan kisaran umur 2 – 2,5 tahun dan bobot badan awal 229,86±12,46 kg. Ternak secara acak dibagi menjadi tiga kelompok, yaitu T₀ diberikan ransum sesuai dengan kebiasaan tradisional penggemukan oleh peternak, T₁ diberikan ransum dengan level 12% PK dan 72% TDN, dan T₂ diberikan ransum dengan level 15% PK dan 72% TDN. Ternak memperoleh pakan secara individu dan air minum secara *ad libitum* selama 90 hari. Data diuji dengan analisis ragam. Hasil penelitian menunjukkan keseimbangan nitrogen, serta urea darah antara T₁ dan T₂ relatif sama, namun lebih tinggi (P<0,05) dibandingkan T₀. Sebaliknya, sintesis protein mikrobia, dan glukosa darah pada 0, 4, dan 6 jam sebelum dan sesudah konsumsi pakan relatif sama diantara ketiga kelompok. Glukosa darah T₂ pada 2 jam setelah konsumsi pakan lebih tinggi (P<0,05) dari T₀ namun relatif sama dengan T₁. Dapat disimpulkan bahwa penggunaan PK 12% dan TDN 72% secara optimal meningkatkan keseimbangan N dan metabolit darah, namun tidak berpengaruh positif terhadap sintesis N dan protein mikrobia.

Kata Kunci: keseimbangan N, N mikrobia, penggemukan, peternakan rakyat, sapi Bali, sintesis protein

ABSTRACT

Research was aimed to determine nitrogen balance, microbial protein synthesis, and blood metabolites of male Bali cattle fattening fed ration with different protein level in smallholder farms North Central Timor, Province of East Timor Tenggara, Indonesia. The cattle used were 18 heads aged 2 to 2.5 years with initial body weight of 229.86±12.46 kg. The cattle were randomly divided into three treatment groups. The T₀ group was given feed the same as traditional fattening cattle practices by farmers, T₁ group fed ration containing 12% crude protein (CP) and 72% total digestible nutrients (TDN), and T₂ group fed ration containing 15% CP and 72% TDN. Cattle were fed individually for 90 days and drinking water *ad libitum*. The data were analyzed by analysis of variance. Results of research indicated the nitrogen balance, and blood urea nitrogen between T₁ and T₂ were relatively similar, but those were higher (P<0.05) than T₀. In contrast, microbial proteins synthesis, and blood glucose at 0, 4, and 6 hours before and after feeding were relatively similar between the groups. Blood glucose of T₂ at

2 hours after intake were higher ($P < 0.05$) than T_0 , but was not different with T_1 . It can be concluded, that the fattening male Bali cattle fed ration containing 12% CP and 72% TDN improved the nitrogen balance and blood metabolites, but it was no positive effect on the microbial proteins and N synthesis.

Keywords : Bali cattle, fattening, microbial nitrogen, Nitrogen balance, protein synthesis, Smallholder farms

INTRODUCTION

Productivity of beef cattle during fattening period was influenced by the feed quality and quantity given by farmers. When the adequately of feed covered during the growth phase, the synthesis of body tissue increases. Such conditions have a positive effect on the body weight gain and carcass production of the cattle.

In West Timor, the fattening of Bali cattle was mostly done in smallholder farms traditionally (Tahuk and Dethan, 2010). As a result, the guarantee of adequate protein and energy to meet the needs of livestock for optimal production is not achieved, especially in the dry season. These was indicated by the low average daily gain (ADG) as well as the poor of feed conversion ratio (Tahuk *et al.*, 2017).

The strategy to ensure the good growth of Bali cattle during fattening period in smallholder farms is improvement of the feed quality. Protein and energy are two important nutrients, which need to be considered in balancing the dietary livestock to increase productivity. When cattle were given high protein with inadequate energy, the inefficient protein utilization may occur (Valente *et al.*, 2016), and microbial protein synthesis is not optimum (Bach *et al.*, 2005). The current concept in ruminant nutrition is focused on maximizing rumen microbial protein synthesis (Zadeh *et al.*, 2013).

The enhancement of microbial proteins synthesis in the fattening ruminant is important, as it contributes greatly to the increase of livestock productivity. Mullik (2006) reported, about 60 to 80% of the total protein required by ruminants originates from the rumen microbial protein. On the other hand, the maximum potential of rumen microbes to produce microbial protein and degraded nutrients in the rumen may be explored only by the provision of high quality diet (Verbic, 2002).

Improving the feed quality on fattened beef cattle is very important to be done by farmers. In vitro observation in the Bali cattle rations that have different levels of protein-energy indicated that microbial protein synthesis has not been

maximal (Tahuk *et al.*, 2016); similarly, microbial protein synthesis in Bali cattle fed different levels of protein and energy were relatively similar (Setiawan *et al.*, 2016).

Nitrogen balance was an indicator to evaluate the adequacy of protein in livestock based on the amount of protein consumed, and which is excreted by livestock (Utomo, 2012). This is due to live weight gains may not be related to protein stored, and a more accurate evaluation of a protein may be obtained by using the results of nitrogen balance experiments (Mc Donald *et al.*, 2011). Therefore, the data of nitrogen balance generally indicates nutrient status in livestock.

According to the above description, then the aims of study was to determine the balance of nitrogen, purine derivates, microbial protein synthesis and efficiency, as well as blood metabolites of male Bali cattle fattened in smallholder farms fed rations with different protein levels.

MATERIALS AND METHODS

Research Location and Bali cattle

The study was conducted in the Nekmese Farmer Group, Usapinonot Village, West Insana District, Regency of Timor Tengah Tenggara, East Nusa Tenggara, Indonesia for 90 days. The chemical composition of feed and feces were analysis in the Nutritional Biochemistry Laboratory, Animal Science Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Male Bali cattle used were 18 heads aged 2-2.5 years with initial body weight (BW) of 229.86 ± 12.46 kg. Cattle were randomized and placed in individual stall equipped with different eating and drinking places. Fattening was done for 90 days, including the adaptation period for 14 days. Feeding was done three times a day at 7:00 am, at 12:00 and 17:00 pm. Drinking water was available ad libitum. Feeding of forage and concentrates were carried out separately. Forage give first, while concentrate was given 60 minutes later after the cattle get forages.

Design of Research and Animal Feed

Exprimental design used was completely

randomized design with three treatments and six replications. The treatments was T₀ group (control), T₁, and T₂ groups. Cattle in the T₀ group obtained varied rations according to the traditional practice of farmers in fattening the cattle. Rations for the group T₁ and T₂ were prepared with different levels of crude protein (CP) with iso-energy in the form of total digestible nutrients (TDN). The group of T₁ get ration containing 12% of CP and 72% of energy (TDN), and the group of T₂ get ration 15% CP, and 72% energy (TDN). Rations used for T₁ dan T₂ group include of native grass, *Gliricidia sepium*, corn meal and rice bran (Table 1 and Table 2). During fattening, livestock also obtain minerals composed of Calcium 165.000 mg, Phosphorous 52.000 mg, Sodium 157 000 mg, Iron 2500 mg, 2500 mg Copper, Manganese 2000 mg, 125 mg Iodine, Cobalt 50 mg, 5000 mg and Selenium Zinc 10 mg. The minerals above its use in livestock were at 2% per kilogram of concentrate.

Variable Measurement and Data Collection

In this study, the observed variables included the nitrogen intake, excretion, and nitrogen balance, purine derivates, microbial protein synthesis, rumen organic matter digestible, and blood metabolites (total plasma protein, hemoglobin, leucocytes, erythrocytes, packed cell volume, blood glukosa and blood urea nitrogen) of male Bali cattle.

Feces collection for determining feed digestibility was conducted over 10 days by using the technique of the total collection (Harris, 1970). Feces were collected daily, weighed, and sprayed with a solution of 10% formalin to avoid decomposition of feces and lost of N feces. The sample was dried until the weight constant; furthermore the samples collected during 10 days was mixed thoroughly, then 10% of the samples were milled with willey mill of 1 mm diameter for laboratory analysis.

Urine collection for the determination of N balance and microbial protein synthesis of purine derivatives were using a harness in conjunction with the implementation of the feces collection (Harris, 1970). The Urine collected from each animal was filtered with a filter cloth to avoid a three-layer mixed with other debris, then the volume weight was weighed and calculated every morning. Urine samples was taken 120 mL and placed in a plastic bottle size of 120 cc/mL, added with 10% of H₂SO₄ until the pH became acidic (pH 2–3), then stored in a freezer at -20°C while

waiting for laboratory analysis.

Nitrogen consumption was calculated from the amount of N in the ration reduced by the remaining N of the ration. The N excretion of feces was calculated by the total feces multiplied by the N content of feces (%). The nitrogen balance was calculated by reducing the N consumption with the N feces and N urine (Mc Donald *et al.*, 2011).

Estimates of microbial protein synthesis in the rumen were measured by the purine derivative method of urine as directed by Chen *et al.* (1992). The calculation of estimation of microbial of nitrogen supply (EMNS) was based on the amount of urine absorbed (X=mmol/day), where the X value was estimated based on excretion of purine derivatives in urine (Y = mmol/day). The supply of N microbial calculation in Bali cattle used the equation according to Yusiaty (2005): $EMNS (g/day) = (X \times 70) / (0.83 \times 0.195 \times 1000)$. The value of X was estimated from the equation $Y = 0.145 W^{0.75} + 0.86 X$ mmol/day (X = purine derivative). The N Purine content was 70 mg/mmol, microbial purine digestibility was 0.83, and ratio N purine with N microbial of Bali cattle was 0.195.

The blood collection was conducted to determine glucose levels and blood urea. Blood sampling was conducted at 0 hours before feeding, and 2, 4 and 6 hours after feeding, which was carried out in the last week of the study. The examination of both variables using spectrophotometer method with reagent kit make DiaSys (according to manufacturer's instructions of DiaSys System GmbH, Holzheim, Germany). Blood glucose level was calculated by the equation: $(Abs. Sample / Abs. Standard) \times 100$ mg/dl, at wavelength 546 nm, while the blood urea nitrogen level was calculated with the equation: $(Abs. Sample / Abs. Standard) \times 50$ mg/dl, at a wavelength of 365 nm). The variable hemoglobin was analyzed by Sahli method, the amount of erythrocytes and leucocytes using Neubauer count method with hemacytometer, total plasma protein with refractometer method, and hematocrit/packed cell volume (PCV) using Wintrobe method.

Identification of chemical feed composition was carried out by proximat analysis according to the AOAC procedure (2005).

Statistic Analysis

The data collected was analyzed according to the one way analysis of variance procedure.

Table 1. The Chemical Composition of Feedstuffs on Male Bali Cattle Fattening with Different Levels of Protein in Smallholder Farms

Feedstuffs	DM (%)	OM	Ash	CP	Extract eter	Crude Fiber	NFE*	TDN**	NDF (%)	ADF (%)
Native grass	36.54	84.71	15.29	6.08	4.11	32.80	41.72	57.30	68.72	39.74
<i>Gliricidia sepium</i>	24.27	87.56	12.44	24.19	12.55	14.51	36.31	72.21	32.98	21.87
<i>Leucaena leucocephala</i>	25.25	90.19	9.81	25.65	8.41	18.96	29.52	79.79	34.11	18.47
<i>Sesbania grandiflora</i>	20.33	85.29	14.71	21.77	8.90	13.18	32.94	75.06	24.72	15.85
<i>Pennisetum purpureoidess</i>	25.24	86.62	13.38	11.98	4.68	31.07	32.37	46.69	68.09	36.24
Banana Stem***	10.40	85.49	14.51	3.31	7.57	32.36	32.08	54.77	47.65	25.85
Banana Leaves	22.12	91.06	15.01	4.37	23.17	8.90	39.87	50.69	-	-
Cassava leaves****	24.42	90.34	9.66	24.52	8.23	19.47	31.16	79.45	-	-
Corn meal	90.09	98.81	1.19	7.89	1.44	1.82	87.66	87.66	22.14	1.99
Rice bran	90.42	84.49	8.26	6.97	2.03	17.37	65.37	65.37	54.67	40.32

DM= dry matter, OM= organic matter, CP = crude protein,

* NFE (nitrogen free extract) = [100 – (% Ash + % CF + % EE + % CP)]

**TDN = According to equation of Harris *et al.* (1972) cited by Hartadi *et al.* (1980); NDF = Neutral detergent fiber, ADF = Acid detergent fiber

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Table 2. The Ration for Fattening Male Bali Cattle with Different Level of Protein in Smallholder Farms

Treatments	Feedstuffs	Composition (% dry matter)	Ration Nutrient Composition (%)	
			CP	TDN
T ₀ *	Forages	100.00	17.09	65.51
	• Native grass	14.00	0.85	9.92
	• Leaves of <i>Gliricidia sepium</i>	30.00	7.26	21.66
T ₁	• Corn meal	39.00	3.08	32.66
	• Rice bran	17.00	1.18	8.61
	Total	100.00	12.37	72.85
T ₂	• Native grass	13.00	0.79	9.21
	• Leaves of <i>Gliricidia sepium</i>	46.00	11.13	33.22
	• Corn meal	28.00	2.21	23.45
	• Rice bran	13.00	0.91	6.58
	Total	100.00	15.03	72.46

*Adjusted with feed provision by the farmers. Forage proportion including native grass 33.76%, *Gliricidia sepium* 26.71%, *Leucaena leucocephala* 26.99%, *Sesbania glandiflora* 3.52%, *Pennisetum purpureoidess* 1.78%, and others feedstuffs 7,24%.

Further trials with Duncan's multiple range test (DMRT) do when there were differences among the treatments (Quinn and Keough, 2002).

RESULTS AND DISCUSSION

Nitrogen Intake, Excretion and Balance

Nitrogen intake (N) (g/head/day; g/kg BW^{0.75}) of cattle in T₁ group was higher than those in T₀ (P<0.05), but those in T₂ was relatively similar to T₁ and T₀. The N feces of T₀, T₁, and T₂ groups was relatively similar. In contrast, the excretion of N in urine (g/head/day) from T₁ and T₂ group was relatively the same, but those were lower (P<0.05) than the T₀ groups (Table 3). The high of N intake in T₁ group was related to the DM and CP intake which is quite high if compared to the T₀ group. This allows cattle to increase their productivity.

The excretion of N feces was relatively similar between the T₀, T₁ and T₂ groups. This was related to CP digestibility which was also not different between the cattle groups (Tahuk *et al.*, 2017). In contrast, urinary N excretion of T₀ cattle group was higher than those of the T₁ and T₂. This condition was caused by inefficient utilization of N because the lack of energy derived from digestible carbohydrates (Gehman and Kononoff, 2010). The cattle of T₀ groups only obtain energy source from structural carbohydrates which have a lower digestibility, so it does not sync with the

high N supply of legumes intake. This study differs from Jetana *et al.* (2010) study which mentions the increased synchronization of carbohydrates and N causes decreased fecal N in Thai Brahman cattle. This condition was probably caused by the differences of livestock genetic and feed quality used in the study.

In the T₀ group the amount of N excretion reached 87.01% of the total N consumed by cattle, where 30.07% N came from the feces, and 56.94% of the urine. Thus, only 12.99% N was utilized to meet livestock needs. This condition illustrates that in T₀ group, the utilization of N feed by livestock for synthesis of body tissue was not maximal. Low digestible carbohydrates as the source of the carbon skeleton is thought to be the trigger for this condition. This was evident from the lower of growth performance on T₀ cattle than the other two treatments. According to Soeparno (2009), the high intake of protein and energy on the livestock will result in a faster of growth rate.

Nitrogen total excretion of T₁ group reached 59.37% from total N intake, consisted of N feces excretion 26.53% and N urine 32.84%. Similarly, total N excretion of T₂ group cattle reached 50.82% of total N intake, which came from N feces 25.37% and N urine 25.45%. This means that in the T₁ and T₂ cattle groups, the use of N by livestock for performance improvement was 40.63 and 49.18%, respectively. The results showed that the use of N feed on the male Bali cattle of T₁ and

Table 3. Nitrogen Balance of Male Bali Cattle Fattening Fed Ration with Different Levels of Protein in Smallholder Farms¹

Variable	T ₀ ²	T ₁ ³	T ₂ ⁴
N intake (g/head/day)	125.31±26.40 ^a	155.40± 8.59 ^b	138.02±11.50 ^{ab}
N intake (g/kgBB ^{0.75} /day)	1.92± 0.34 ^a	2.24± 0.16 ^b	2.04± 0.09 ^{ab}
N of feces ^{ns} (g/head/day)	37.68± 4.40	41.22± 3.16	35.02± 7.52
N of feces ^{ns} (g/kgBB ^{0.75} /day)	0.59± 0.07	0.59± 0.05	0.52± 0.10
N of urine (g/head/day)	71.35±23.49 ^b	51.03±14.15 ^a	35.12± 4.87 ^a
N of urine (g/kgBB ^{0.75} /day)	1.10± 0.36 ^b	0.74± 0.21 ^a	0.52± 0.08 ^a
N balance (g/head/day)	16.29±33.34 ^a	63.14±20.18 ^b	67.89±11.57 ^b
N balance (g/kgBB ^{0.75} /day)	0.24± 0.50 ^a	0.91± 0.29 ^b	1.00± 0.14 ^b

¹Data is presented in average ±SD

²T₀=control (according to the traditional raising of farmers); ³T₁=ration with 12% CP and TDN 72%;

⁴T₂=ration with 15% CP and 72% TDN; ^{ns}= not significantly different;

^{a,b}Different superscript in the same row shows differences at P<0.05.

T₂ group was better if compared to T₀ group. The lower N excretion value of T₁ and T₂, as well as the growth performance of the two treatments was higher than those of T₀, where the ADG of T₁ and T₂ were 0.70 and 0.47 kg/head/day, respectively (Tahuk *et al.*, 2017).

Nitrogen balance in male Bali cattle fattening groups of T₁ and T₂ were not significantly differ, but those were higher (P<0.05) than T₀ group (Table 3). The results of this study showed that the improvements feed in T₁ and T₂ groups can increase consumption and digestibility of CP or N, which in turn had a positive impact towards N balance. According to Yusiati (2005), the low N intake, contributes to the decrease of N balance in male Bali cattle.

The N balance of the three groups was positive in the study. This can be seen from the increase of ADG of T₁ and T₂ groups, each of which reaches 0.70±0.16, and 0.47±0.47 kg/head/day (Tahuk *et al.*, 2017). The T₀ group showed a smaller balance value and tended to approach a value of 0. This situation illustrated the use of CP or N was less than the maximum for the cattle growth, indicated by ADG which only reaches 0.30±0.05 kg/head/day (Tahuk *et al.*, 2017). Instead, the group of T₁ and T₂ had the equilibrium value of N that was higher from value 0, which described high deposit of N to improve the performance of cattle production. According to Howard *et al.* (2007), rations composed of carbohydrates and N synchronized have a positive effect on improving N balance. When nitrogen balance means was positive, it means cattle will increase body weight due to the addition of the woven tendons (Mc Donald *et al.*, 2011).

Purine Derivates

The content of creatinine, uric acid, allantoin and purine derivatives (mmol/l) in male Bali cattle fattening were not significantly different between groups (Table 4). Thus, the treatment of CP levels differing by 12 and 15% in 72% TDN, generally did not positively affect to the increase secretion of purine derivatives.

According to Yusiati (2005), purine derivatives are influenced by species of livestock and the amount of feeding. In addition, Bali cattle generally have lower purine derivatives of other local beef cattle in Indonesia, such as PO and PFH cattle. Similarly, purine derivatives increased in Bali cattle that received feed *ad libitum* than livestock given limited feed .

Estimate Microbial Protein and N Synthesis of Purine Derivatives

Estimation of microbial N synthesis (g/head/day) of purine derivatives in group T₀, T₁, and T₂ was 7.22±1.94; 8.09±3.23; and 8.51±1.01, respectively; whereas estimation of microbial protein synthesis of T₀, T₁, and T₂ was 45.12±12.09; 50.55±20.21, and 53.20±6.29, respectively (Table 4). The estimation of microbial N and protein synthesis of purine derivatives in the three groups showed no significant difference. This is related to the secretion of purine derivatives from the three groups of cattle that were also relatively similar. Those indicated that the use of feed containing 12 and 15% CP and 72% TDN had no significant impact on the increase in the N and microbial protein synthesis compared to the use of forage as a single feed on fattening cattle in smallholder farms. The results of this study were not different from *in vitro* study by Tahuk *et al.* (2016) which obtained relatively the same of microbial protein synthesis in fattening rations of male Bali cattle based on different protein and energy levels. However, the synthesis of microbial proteins in this study was lower than that of Bali cattle that obtained rations with multivitamins and minerals supplementation (Mudita *et al.*, 2014).

Theoretically, livestock given high CP and TDN can improve the microbial proteins synthesis in the rumen. In fact, microbial protein synthesis in the group T₁ dan T₂ was not optimal. These were allegedly because associated with the lack synchronization of nitrogen and energy in the rumen.

The study were in accordance with the report of Devant *et al.* (2001), which declared the use of N sources in rations of high concentrate and low protein in beef cattle had sufficient N available for microbial growth, but limited to microbial protein synthesis and digestion of nutrients. Pathak (2008) stated that a crude protein content in rations arranged higher than 11% may be necessary to assist optimal microbial growth; but because of it resistance protein to microbial degradation may result in limited microbial protein synthesis. Therefore, to increase the amount of microbial synthesis, the manipulation of energy and N fermentation in the rumen is necessary to obtain an energy supply pattern appropriate to the rumen conditions.

Rumen Organic Matter Digestible

Rumen organic matter digestible (ROMD) of

Table 4. Microbial N and Protein Synthesis of Male Bali Cattle Fattening Fed Ration with Different Levels of Protein in Smallholder Farms¹

Variable	T ₀ ²	T ₁ ³	T ₂ ⁴
Creatinine (mmol/day) ^{ns}	34.64±10.91	65.54±46.56	30.93±14.09
Uric acid (mmol/day) ^{ns}	0.44± 0.31	0.46± 0.48	0.66± 0.35
Allantoin (mmol/day) ^{ns}	8.03± 5.13	9.55± 8.23	10.82± 2.45
Purine derivatives (mmol/day) ^{ns}	8.47± 5.40	10.02± 8.71	11.48± 2.75
EMPS ⁵ (g/day) ^{ns}	45.12±12.09	50.55±20.21	53.20± 6.29
EMNS ⁶ (g/day) ^{ns}	7.22± 1.94	8.09± 3.23	8.51± 1.01
ROMD ⁷ (kg/day)	1.47± 0.19 ^a	2.99± 0.13 ^c	2.28± 0.17 ^b
Efficiency MPS (g/kg.ROMD/day)	30.80± 6.86 ^b	16.72± 5.93 ^a	23.38± 2.99 ^a
Efficiency MNS (g/kg.ROMD/day)	4.93± 1.10 ^b	2.68± 0.95 ^a	3.74± 0.48 ^a

¹Data is presented in average ±SD;

²T₀= control (according to the traditional raising of farmers); ³T₁= ration with 12% CP and TDN 72%;

⁴T₂= ration with 15% CP and 72% TDN; ⁵EMPS : Estimation microbial protein synthesis (EMPS);

⁶EMNS: Estimation microbial nitrogen synthesis (EMNS);

⁷ROMD (rumen organic matter digestible.obtained) from OM intake (kg) x OM digestible (%) x 65%;

^{ns} = not significantly different;

^{a.b.c}Different superscript in the same row shows differences at P<0.05.

the T₁ group was higher (P<0.05) than those of T₂ group; similarly, the T₂ was higher (P<0.05) than T₀. Despite having a lower ROMD, livestock in the T₀ group was more efficient in utilizing the organic matter consumed and digested in the rumen for microbial synthesis. This can be seen from the efficiency of EMNS and estimation microbial of protein supply (EMPS from ROMD of T₀ group (g/kg.ROMD/day) that was higher (P<0.05) than that of T₁ and T₂ groups. In contrast, the efficiency of EMNS and EMPS values of ROMD in the T₁ and T₂ groups were relatively similar.

The feed proportion of the T₀ group consisted of grasses as a source of structural carbohydrates, and forage leguminous as a source of N was suspected of slow digestion. As a result, the duration of staying in the rumen was longer so that the chance of microbes to degrade was greater than T₁ and T₂ group. This condition has a positive impact on the increase of N synthesis and microbial protein rumen of each organic matter (kg). According to Hasson (2004), when the passage rate increase, the time for digestion in the rumen decreased, this results in a less efficient degradation. At higher feeding level the rumen

outflow increase and this depresses fibre degradation.

In the T₁ and T₂ groups, the efficiency of protein and N microbial synthesis was lower than T₀ group due to lack of synchronization between proteins and digestible carbohydrates. Microbial protein synthesis can be increased if there is synchronization between protein and energy (Zadeh *et al.*, 2013).

Blood Metabolites

Blood components status (hemoglobin, leukocytes, erythrocytes) of male Bali cattle in T₂ group was higher (P<0.05) compared to T₀, but T₁ was relatively similar to both groups. The packed cell volume (PCV) of T₁ and T₂ was relatively similar, but it was higher (P<0.05) than those of T₀; whereas the variable of total plasma protein did not significantly differ between the treatments.

Although there were differences, the general status of blood components was under normal conditions. Thus, the body's physiological function was in a good condition, both from the aspect of metabolism, O₂ transport, and nutrients, as well as optimal immune function. The main

function of blood is to maintain the body's physiological balance (Radkowska and Herbut, 2014). Therefore, the hematological blood indicators are the main determinant of the animals' environmental adaptation and thus can improve welfare livestock (Sattar and Mirza, 2009).

Blood Glucose

Blood glucose concentrations of male Bali cattle showed no significant difference in 0 hours before feeding, as well as 4 and 6 hours afterwards. However, at 2 hours after feeding, blood glucose of T₂ group was higher (P<0.05) than those of T₀, while between T₀ and T₁, and T₁ and T₂ showed no significant difference (Table 5). Increased blood glucose status at 2 hours after feeding was closely related to the level of feed digestibility and availability of precursor glucose in the form of volatile fatty acids (VFA) that were

absorbed into the blood which would then be converted into blood glucose in the liver (Mc Donald *et al.*, 2011). Glucose status at 0 hours before feeding as well as 4 and 6 hours after feeding was normal because of the guard system blood glucose levels in ruminants through a mechanism or process of glycogenesis, glycogenolysis, and gluconeogenesis (Soeparno, 2011).

The results of this study were not much different from the reports of Tahuk *et al.* (2017) who obtained blood glucose levels in male Bali cattle fattening in the smallholder farms with forage was 58.62 mg/dL. Nevertheless, the status of blood glucose in this study was lower than Kendran *et al.* (2012) who obtained blood glucose in male and female Bali cattle were 68.96 – 72.81 mg/dl; and 65.85 to 68.91 mg/dl, respectively; as well as in young and mature Bali cattle that were 68.96-72.51 mg/dL, and 65.68-68.91 mg/dl,

Table 5. Blood Metabolites of Male Bali Cattle Fattening fed Ration with Different Levels of Protein in Smallholder Farms¹

Variable	T ₀ ²	T ₁ ³	T ₂ ⁴
TPP ⁵ (g/dl) ^{ns}	7.90± 0.56	7.20± 0.46	7.63± 0.94
Hemoglobin (g/dl)	14.03± 0.35 ^a	14.97± 0.99 ^{ab}	15.37± 0.80 ^b
Leukocytes (μL)	6,808.33±215.45 ^a	7,858.33±1157.33 ^{ab}	8,800.00±969.54 ^b
Erythrocytes (10 ⁵ μL)	70.52± 3.27 ^a	81.72± 14.39 ^{ab}	85.13± 10.39 ^b
PCV ⁶ (%)	42.03± 1.35 ^a	44.93± 2.89 ^b	46.06± 2.42 ^b
Blood glucose (mg/dL)			
0 hours ^{ns}	55.61±4.18	56.51± 3.11	56.73± 3.12
2 hours	56.45±3.77 ^a	57.47± 2.98 ^{ab}	60.43± 1.60 ^b
4 hours ^{ns}	54.21±4.57	54.15± 2.95	56.39± 4.05
6 hours ^{ns}	59.10±1.73	57.94± 1.97	59.06± 2.16
Blood urea nitrogen (mg/dL)			
0 hours	22.78±3.47 ^a	29.17± 1.08 ^b	28.31± 1.08 ^b
2 hours	22.83±4.14 ^a	28.88± 1.84 ^b	27.38± 1.22 ^b
4 hours	19.74±3.80 ^a	27.43± 1.27 ^b	27.33± 0.92 ^b
6 hours	22.12±3.06 ^a	29.87± 1.25 ^b	29.73± 2.32 ^b

¹Data were presented in average ±SD

²T₀=control (according to the traditional raising of farmers); ³T₁= ration with 12% CP and TDN 72%;

⁴T₂=ration with 15% CP and 72% TDN; ⁵TPP = total plasma proteins; ⁶PCV = packed cell volume

^{ns}= not significantly different;

^{a,b}Different superscript in the same row shows differences at P<0.05.

respectively.

Blood Urea Nitrogen

Concentration of blood urea nitrogen (BUN) (mg/dL) of male Bali cattle at 0 hours before feeding or 2, 4, and 6 hours after feeding in T₁ and T₂ groups was higher (P<0.05) compared to those in T₀ (Table 5). Cattle in T₀ group had lower blood urea concentration than the normal range. It was presumably related to low N-NH₃ concentration, so the conversion to blood urea nitrogen was lower. The normal range of blood urea in cattle were in accordance to Hungate (1966) cited by Munzaronah *et al.* (2010) was 26.6-56.7 mg/dL. On the other hand, blood urea in T₁ and T₂ groups was higher than those in T₀ because of the high concentration of N-NH₃ rumen, while the conversion to microbial protein was lower.

The efficiency of N-NH₃ utilization for protein synthesis in the rumen depended on the availability of energy. If there was a shortage of energy, then the protein would be excessive and cannot be utilized by rumen microbes. The excess of crude protein can increase the concentration of urea in the plasma (Mc Donald *et al.*, 2011). The BUN content in these study was lower than those in Madura cattle fed ration containing different energy levels (Umar *et al.*, 2015). In contrast, the content of BUN in the T₁ and T₂ treatment was higher than those in Tahuk *et al.* (2017).

CONCLUSION

The fattened male Bali cattle fed ration with level 12% CP, and 72% TDN can improve the nitrogen balance and blood metabolites, but it was no positive effect on the microbial proteins and N synthesis.

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