Nutrient Utilization, Body Composition and Lactation Performance of First Lactation Bali Cows (*Bos sondaicus*) on Grass-Legume Based Diets[a]

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ABSTRACT : A study on energy and protein utilization, and milk production of Bali cows on grass-legume diets was carried out using 12 first lactation cows (initial BW 263.79±21.66 kg) during a period of 16 weeks starting immediately post calving. The animals were randomly allotted into 4 dietary treatment groups R1, R2, R3 and R4, receiving from the last 2 months of pregnancy onwards, graded improved rations based on a mixture of locally available grass and legume feed *ad libitum*. R1 contained on a DM basis 70% elephant grass (PP, *Penisetum purpureum*) plus 30% *Gliricidia sepia* leaves (GS), R2 was 30% PP plus 55% GS supplemented with 15% *Hibiscus tilliactus* leaves (HT, defaunating effect), R3 and R4 were 22.5% PP+41.25% GS+11.25% HT+25% concentrate, where R3 was not and R4 supplemented with zinc di-acetate. TDN, CP and zinc contents of the diets were 58.2%, 12.05% and 18.3 mg/kg respectively for R1, 65.05%, 16.9% and 25.6 mg/kg respectively for R2, 66.03%, 16.71% and 29.02 mg/kg respectively for R3 and 66.03%, 16.71% and 60.47 mg/kg respectively for R4. Milk production and body weight were monitored throughout the experimental period. *In vivo* body composition by the urea space technique validated by the body density method and supported by carcass data was estimated at the start and termination of the experiment. Nutrient balance and rumen performance characteristics were measured during a balance trial of 7 days during the 3rd and 4th week of the lactation period. Results indicated that quality of ration caused improvement of ruminal total VFA concentration, increments being 52 to 65% for R2, R3 and R4 above R1, with increments of acetate being less (31 to 48%) and propionate being proportionally more in comparison to total VFA increments. Similarly, ammonia concentrations increased to 5.24 to 7.07 mM, equivalent to 7.34 to 9.90 mg NH_3-N/100 ml rumen fluid. Results also indicated that feed quality did not affect DE and ME intakes, and heat production (HP), but increased GE, UE, energy in milk and total retained energy (RE total) in body tissues and milk. Intake-, digestible- and catabolized-protein, and retained-protein in body tissues and milk (Rprot) were all elevated increasing the quality of ration. Similar results were obtained for milk yield and components with mean values reaching 2.085 kg/d (R4) versus 0.92 kg/d (R1) for milk yield, and 170.22 g/d (R4) vs 71.69 g/d (R1), 105.74 g/d (R4) vs 45.35 g/d (R1), 101.34 g/d (R4) vs 46.36 g/d (R1) for milk-fat, -protein, and -lactose, respectively. Relatively high yields of milk production was maintained longer for R4 as compared to the other treatment groups. There were no significant effects on body mass and components due to lactation. From the relationship RE_{total} (MJ/d)=12.79-0.373 ME (MJ/d); (r=0.73), it was found that ME_m=0.53 MJ/kg$W^{0.75}$.d. Requirement of energy to support the production of milk, ranging from 0.5 to 3.0 kg/d, follows the equation: Milk Prod. (Q_{mp}, kg/d)=[-2.48+4.31 ME(MJ/kg$^{0.75}$.d)]; (r=0.6) or Q_{mp}=-3.4+[0.08(ME-$RE_{body\ tissue}$)]MJ/d]; (r=0.94). The requirement for protein intake for maintenance (IP_m) equals 6.19 g/kg$^{0.75}$.d derived from the relationship RP=-47.4+0.12 IP; (r=0.74, n=9). Equation for protein requirement for lactation is Q_{nl}=[(Q_{mp})(% protein in milk)(I_{mp})]/100, where Q_{nl} is g protein required for lactation, Q_{mp} is daily milk yield, Bali cow's milk-protein content av. 5.04%, and I_{mp} is metabolic increment for milk production (ME_{lakt}/ME_m=1.46). (***Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1681-1690***)

Key Words : Bali Cattle, *Bos sondaicus, banteng*, Energy and Protein Requirements, Lactation, Milk Yield, Composition

INTRODUCTION

Bali cattle have resulted from the domestication of the wild Banteng of the humid tropics, a breed that has truly native origins in Indonesia and has remained purebred on the island of Bali. Compared to other breeds of cattle, Bali cattle have good fertility and can survive on poor pasture. Although they have slower growth rates than Ongole cattle, their reproductive performance is thought to be better. They also have better tick resistance and are easier to handle. Banteng cattle appear to tolerate external as well as internal parasites. They are reported to be preferred by small holders as the draught breed of animal. Another remarkable feature of this cattle is the ability to thrive under hot, humid and disease ridden conditions where other breeds of cattle often grow poorly (International

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Embryos Ltd., 1986).

There is little information on the biology of the Bali cattle. Cows are small in size averaging 250 kg in weight. They are late maturing and rarely produce a calf before 4 years of age, but this situation may be due to poor feeding early in life. At birth, males weigh about 16 to 17 kg and females about 14 to 15 kg. Calves are not weaned at any specific time, and dams may continuously nurse a calf until the next birth. Potential advantages of Bali cattle include their ability to utilize low quality feeds and they can live off forage unpalatable to temperate cattle breeds. Bali cows seem also to lose less weight and condition during lactation which may be due to their lower milk production.

Bali cows are poor milk producers with almost invisible udders and a lactation varying from 6 to 10 months producing 0.9-2.8 kg per day. This situation causes slower growth and even great mortality of calves during the pre-weaning period (Soehadji, 1991), especially for calves born during the dry season under extensive management. Wirdahayati et al. (1998) reported juvenile mortality ranging from 20 to 45% annually. Liwa (1992) found that growth rate of calves up to 120 days of age were greatly influenced by milk production and that in turn depends on the nutritional status of the dam. Weaning weights of calves are 60% influenced by milk production of the dam and 40% by genetic traits (Rutledge et al., 1971). Bali cattle are also prone to certain diseases like the "jembrana", "bali-ziekte", and their high susceptibility to bovine malignant catarrhal fever.

Among the factors affecting the productivity of Bali cattle, the most propitious methods for improvement are in feeding and nutrition. Thus, in judgment to the objective of better performance, efforts must be made to improve the prevailing feeding and management practices. The present paper reports results of a study to evaluate the response of the lactating Bali cow in producing milk by feeding her improved diets based on a mixture of grass and legume, with or without supplements. The experimental cows were taken from a heard reared by farmers supervised by the Accelerated Bali Cattle Development and Breeding Project (P3 Bali at Pulukan, Bali) which were of better condition than Bali cattle reared under traditional smallholder farming where a critical body live weight of 230 kg was prevalent (Winugroho and Teleni, 1987).

MATERIALS AND METHODS

Animals and feeding

Twelve first lactation Bali cows [initial body weight (BW) 263.79±21.66 kg] obtained from the Accelerated Bali Cattle Development and Breeding Project (P3 Bali at Pulukan, Bali), were used immediately after calving for a 16 week nutritional and milk production experiment. The experiment adopted a complete randomly group design with 4 ration treatments and 3 blocks as replicates where each replicate was represented by a single cow. The rations R1, R2, R3 and R4 composed of locally available feed stuffs, were offered *ad libitum*. R1 contained on a DM basis 70% elephant grass (PP, *Penisetum purpureum*) plus 30% *Gliricidia sepia* leaves (GS), R2 was 30% PP plus 55% GS supplemented with 15% *Hibiscus tilliacius* leaves (HT, defaunating effect), R3 and R4 were 22.5% PP+41.25% GS+11.25% HT+25% concentrate, where R3 was not and R4 was supplemented with zinc di-acetate. TDN, CP and zinc contents of the diets were 58.2%, 12.05% and 18.3 mg/kg respectively for R1, 65.05%, 16.9% and 25.6 mg/kg respectively for R2, 66.03%, 16.71% and 29.02 mg/kg respectively for R3 and 66.03%, 16.71% and 60.47 mg/kg respectively for R4. The cows were accustomed to the diets since 1 to 2 months before calving. Details of the composition of the rations are given in table 1.

Using a 600 kg maximum capacity Rudweigh (Guyra, NSW, Australia) digital scale, the cows were weighed bi-weekly in the morning for 3 consecutive days and the average value was taken. Weighing of cows and calves started within 24 h after parturition.

The calf was allowed to feed on colostrum milk during the first week and subsequently, starting from the second week onwards, the cows were hand milked twice daily following injection of oxytocin (1.0 U); daily yields were recorded throughout the experimental period. In the 3rd to 4th week of the experiment, the animals were placed in individual pens for balance trials lasting 7d. Excreta and refused feed were wholly collected before morning feeding, weights recorded, and sampled daily for composite proximate analysis. For this purpose, daily samples of 500 g forage and 200 g concentrate were used. Feces samples were 5% of daily excretion conserved in chloroform (0.5% of sample weight) (Juko et al., 1961) while urine samples for urinary N determination required the addition of concentrated HCl at a level of 2% of urine volume (v/v). Daily aliquots of milk were also taken for composite milk analysis. Energy contents of feed and feces were determined by bomb calorimetry using a Gallenkamp calorimeter. Milk protein was estimated from N content analyzed by the micro-kjeldahl method and multiplied by 6.38, milk fat and lactose were estimated according to AOAC (1970). Milk energy was calculated by summation of energies in milk protein, fat and lactose, using respectively the heat of combustion values of 23.85, 38.50 and 16.74 kJ/g.

Ruminal fermentation

By the end of the balance trial, rumen fluid from each cow was taken by way of a stomach tube for

Table 1. Nutrient composition of rations for experimental lactating Bali cows

Feed ingredient/nutrient		R1	R2	R3	R4
		---------- % of ration DM ----------			
Elephant grass *Pennisetum purpureum*		70	30	22.5	22.5
Gliricidia sepium legume		30	55	41.25	41.25
Hibiscus tilliacius leaves (defaunating)		-	15	11.25	11.25
Concentrate ingredient:					
Rice bran		-	-	10.053	10.083
Coconut cake		-	-	12.150	12.150
Corn oil		-	-	1.35	1.345
Salt		-	-	0.617	0.617
Supercalc mix		-	-	0.193	0.193
Ammonium sulfate		-	-	0.607	0.607
Zinc acetate		-	-	-	0.005
Total		100	100	100	100
Nutrient content:	TDN (%)	58.2	65.05	66.03	66.03
	CP (% DM)	12.05	16.90	16.71	16.71
	Gross energy (MJ/kg)	16.29	17.02	16.75	16.75
	Zn (mg/kg)	18.3	25.6	29.02	60.47

measuring ruminal characteristics. Ruminal fluid pH was measured using an Orion model 250 Fisher pH meter. Ruminal NH_3 concentration was determined by the diffusion method of Conway (General Laboratory Procedures, 1966). Rumen microbial protein synthesis was estimated according to Shultz and Shultz (1969), in which 20 ml rumen fluid was mixed with 5 ml H_2SO_4 1.07 N, and 5 ml 10% sodium tungstate solution and the mixture allowed to stand for 4 h. The mixture was subsequently centrifuged at 5000 rpm for 20 min. The precipitate was washed with a mixture of distilled water, H_2SO_4 and tungstate 4:1:1, re- centrifuged, and the precipitate rewashed and centrifuged again; the final precipitate was then analyzed for DM (A mg) and CP by semi-micro Kjeldahl (B% of DM). Thus microbial protein of the precipitate (C, mg/20 ml) was calculated as (B× A)/100 and, subsequently, rumen microbial synthetic rate (mg/liter.h) was found as (C×50)/3. Individual VFAs in rumen fluid were measured by gas chromatography. Total VFA was found by summation of acetate, propionate and butyrate. Rumen protozoal counts were determined by a staining method using Trypan Blue Formaline Saline (Suryahadi, 1990) involving counting protozoa using a counting chamber and viewed under a light microscope with 100× magnification. The protozoal count in 1ml rumen fluid (P) was found according to: P=(100×C×DF)/(0.2× 0.0625×16×16), where C=numbers of protozoa counted in the counting chamber and DF=dilution factor. Bacterial count in rumen fluid was estimated by counting colonies of live bacteria after rumen fluid sample was serially diluted and cultured using non-selective media at pH=7, 39℃, and anaerobic conditions (flushing with CO_2) in Hungate tubes for 7 days (Suryahadi, 1990).

Body composition and measurement

Body components were measured from urea space data obtained by the method according to Rule et al. (1986), using prediction equations for body-water and -fat, originally developed for dairy cattle. Briefly, a 30% urea in saline solution at a dose of 0.46 ml per kg $BW^{0.75}$ was intravenously injected via *v.jugulares*. Exactly 12 minutes after urea injection, a sample of blood was withdrawn for plasma urea analysis and the increment of plasma urea from the pre-injection value, ΔU, was used for the calculation of urea space (US). US of the cow was calculated according to equation US=[mg urea injected]/[10×BW×mg ΔU]. Original equations for body water and body fat were: Body water (%BW)=59.1+0.22 US-0.04 BW, and Body fat (%BW)=97.8-1.27 US. The equation for body fat to be applied for the Bali cow was validated by comparing the obtained values with results from body fat determination by the *in vivo* body density method according to the concept developed by Kleiber (1961) as applied by Mahardika et al. (2000) for swamp buffalo. To overcome resistance by the Bali cow during immersion in water, a stanchion on wheels was used for fixing and restraining the animal in a standing position, the stanchion with animal was carefully lowered in a concrete basin constructed in the ground measuring 2.0 m (length)×1.0 m (width)× 1.5 m (depth) having a sloping entrance. The basin was then filled with water until the animal was immersed up to the nostrils. Subsequently, the stanchion with the animal was removed resulting in

lowering of water level. Water was then added until original level and the quantity of water added was equal to the volume of animal plus stanchion. The animal's body volume was found by subtracting this value by the volume of the stanchion. It was found from 15 determinations of cows [213.2 ± 38.18 (SD) kg BW] that the prediction equation for body fat content had to be corrected as %Fat=-0.69+0.89 (%Fat from US); r=0.58 (n=15). The series of values of fat content measured by US and those measured by the body density method were tested by student t test and proven to be significantly different ($p<0.01$). Figure 1 shows the relationship between body fat content measured *in vivo* by the two methods. Body protein was calculated using an equation which relates the ratio of meat (Y, kg) to BW (X, kg), according to equation Y=0.895+0.354 X; r=0.94 (n=15), derived from carcass data collected from the slaughterhouse; subsequently body protein was calculated by multiplying mean protein percentage of Bali cow's meat to quantity of meat. Protein content of the meat samples was determined in the university's meat laboratory and a mean value of 15.492 ± 0.416% (SD) was found. Figure 2 depicts the relationship between meat content and BW of Bali cows.

From the changes in body-protein and -fat measured at the start and immediately after termination of the 16 wk experimental period, Rprotein, Rfat and $RE_{body\text{-}tissues}$ were calculated. $RE_{body\text{-}tissues}$ was found assuming energy equivalents of 39.32 MJ/kg and 20.07 MJ/kg for body-fat and -protein, respectively (Mahardika et al., 1997).

Calculations

For the energy balance calculations, energy loss via ruminal methane production was estimated to be 7.75% of GE intake (Waiman et al., 1980) and urinary energy loss (UE) as catabolized-protein times 5.44 kJ/g protein, which factor was computed from the difference between the *in vitro* and *in vivo* heats of oxidation of protein. (Brody, 1945). Catabolized protein may also be calculated as digested protein minus retained protein in milk and body tissues, while digested protein (DP) was calculated from intake protein (IP) multiplied by % protein digestibility. Retained protein (RP) and retained energy (RE_{total}) values included milk-protein (RP_{milk}) and energy (RE_{milk}), respectively. Metabolizable energy (ME) was calculated from DE minus the sum of energy losses in methane and urine. HP (heat production) was estimated as the difference between ME and RE_{total}.

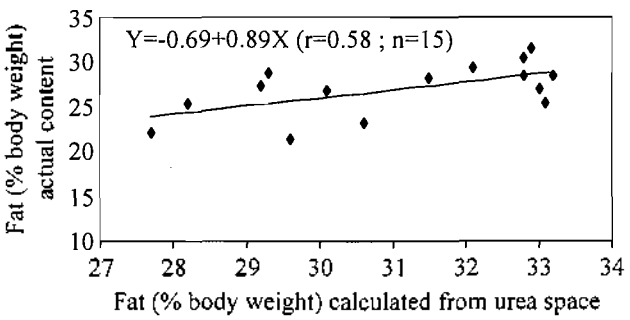

Figure 1. Relation between actual body fat content (measured by body density) and fat calculated from urea space (Rule et al., 1986) in adult Bali cows. The data on actual body fat content are significantly different ($p<0.01$) from the data on fat content obtained with the US procedure.

Statistical analysis

The significance of difference between means was compared using Duncan's Multiple Range Test after ANOVA for one way classified data (Steel and Torrie, 1986). Mathematical models relating measured variables were obtained by regression analysis using Lotus 123 program.

RESULTS AND DISCUSSION

Management practice of Bali cattle at the village level consists of stall feeding with cut forage and grazing during rest periods from draught work. The nutritive value of tropical grasslands, however, tends to be low with high rates of growth and lignin content. The high growth rates may result in a reduced content of essential elements leading to deficiency diseases. The present experiment embraced the grass-legume mixture diet as base ration (ration R1) and successive improvements were made with the other rations R2, R3 and R4 by defaunation, supplementation with concentrate and addition of zinc diacetate, respectively. The defaunating effect of *Hibiscus* leaves were attributed to its saponin content which would kill rumen protozoa (Jalaludin, 1994). The addition of zinc would accomplish as component of many metallo-enzymes (Tillman et al., 1986), pancreatic hormones (Granner, 1987), synthesis of nucleic acids and

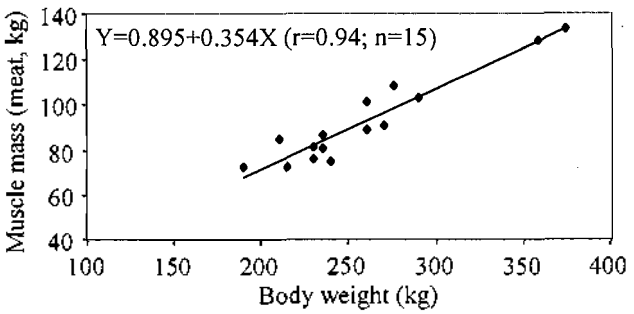

Figure 2. Relation between body muscle mass (meat) and body weight of adult Bali cows

proteins (Lieberman and Bruning, 1990) and carbohydrate metabolism (Church and Pond, 1982).

The results on nutrient intakes and ruminal characteristics are presented in table 2 and those on nutrient digestion and utilization, and milk yield and components in table 3. In general, all data supported the expectation of better response by the improved diets. The average DM intake of all groups ranged from 86.5 to 97.4 g/$W^{0.75}$.d, but differences among treatment groups were insignificant. Significant differences were obtained with protein, fat and energy intakes. These results were attributed to the different nutritional compositions of the rations and may indicate better palatability of the improved rations. Taking R1 as base level, protein intake for R2 increased by 58%, while for R3 and R4 the increments were 46%. Following the same order the increments for fat intake were 42.4% and 152%, and for energy intake the values were 17.7% and 7.8%. As stated earlier, these differences were attributed to the different nutrient composition of the test diets, R1 being the lowest in CP and energy contents, while R2 had the highest CP content. Diets R3 and R4 were supplemented with concentrate, which contained residual corn oil, rice bran and pressed cakes from oil-rich feeds causing relatively higher fat contents. The favorable responses on intakes with the improved diets were also supported by ruminal digestion data reflecting improved feed acceptance. Ruminal fermentation data demonstrated that partial and total concentrations of VFAs were increased for R2, R3 and R4 in comparison to R1, indicating a favorable supply of this primary source of energy for the ruminant animal. Total VFA concentration increased by 52 to 65% for R2, R3 and R4, with acetate increments being less (31 to 48%) while propionate increments being proportionally more (or doubled) in comparison to total VFA concentration increments. Ration R1 gave rise to mixtures of VFAs which is typical of a forage diet, while R2, R3 and R4 with their relatively higher proportions of propionate and butyrate were typical for a moderate level CP of concentrate-supplemented forage diet (Thomas and Rook, 1981). The molar concentrations of the acids in

Table 2. Nutrient intakes, digestion and ruminal characteristics 3 hours post feeding in first lactation Bali cows fed grass-legume based diets

Nutritional parameter	Ration R1	Ration R2	Ration R3	Ration R4	Statistical sign. test	SEM
Intakes and digestion:						
DM intake (g/$W^{0.75}$.d)	86.52^{a}	97.43^{a}	90.94^{a}	90.96^{a}	NS	3.571
Protein intake (g/$W^{0.75}$.d)	10.42^{a}	16.47^{b}	15.21^{b}	15.26^{b}	p<0.01	0.038
Fat intake (g/ $W^{0.75}$.d)	2.05^{a}	2.923^{b}	5.157^{c}	5.17^{c}	p<0.01	0.103
Energy intake(MJ/$W^{0.75}$.d)	1.445^{a}	1.657^{c}	1.524^{b}	1.528^{b}	p<0.05	0.039
Fecal energy (MJ/$W^{0.75}$.d)	0.527^{ab}	0.64^{c}	0.571^{bc}	0.485^{a}	p<0.01	0.013
Methane energy (MJ/$W^{0.75}$.d)	0.113^{a}	0.128^{b}	0.118^{ab}	0.118^{ab}	p<0.01	0.002
Digestible energy (MJ/$W^{0.75}$.d)	0.918^{a}	1.017^{a}	0.953^{a}	1.043^{a}	NS	0.078
Digestible protein (g/$W^{0.75}$.d)	7.27^{a}	12.33^{b}	11.58^{b}	12.02^{b}	p<0.01	0.275
Ruminal characteristics:						
pH	6.5^{a}	6.67^{a}	6.63^{a}	7.17^{a}	NS	0.20
Ammonia (mM)	2.63^{a}	5.24^{b}	7.07^{c}	6.27^{bc}	p<0.01	0.47
Total VFA (mM)	56.41^{a}	87.68^{b}	85.96^{b}	92.99^{b}	p<0.05	5.54
Acetate (mM)	42.72^{a}	60.49^{b}	55.95^{b}	63.11^{b}	p<0.05	3.89
Propionate (mM)	9.38^{a}	19.18^{b}	21.43^{b}	21.35^{b}	p<0.01	1.77
Butyrate (mM)	4.31^{a}	8.01^{b}	8.58^{b}	8.53^{b}	p<0.05	0.66
NGR	5.52^{a}	4.02^{b}	3.46^{b}	3.80^{b}	p<0.01	0.27
Efficiency (%)[1]	71.66^{a}	74.23^{a}	75.76^{b}	74.75^{a}	p<0.05	0.58
Microbial protein prod. (mg/d)	498.74^{a}	623.65^{b}	655.68^{b}	660.15^{b}	p<0.01	9.57
Bacterial count ($\times 10^{7}$ col/ml)	6.14^{a}	8.55^{b}	9.24^{b}	17.28^{c}	p<0.05	0.35
Protozoal count (10^{4} cell/ml)	8.83^{a}	7.36^{a}	21.0^{b}	20.0^{b}	p<0.01	1.13

R1=70% elephant grass (PP, *Penisetum purpureum*+30% *Gliricidia sepium* legume (GS) diet; R2=30% PP plus 58% GS supplemented with 12% *Hibiscus tilliacius* (HT) leaves; R3=R2+concentrate; R3=22.5% PP+43.5% GS+9% HT+25% concentrate, and R4=R3+Zn diacetate (see text for details).

Values in a row with differing letter superscripts differ significantly at p level indicated.

SEM=standard error of the means due to feeding treatment.

DM=dry matter.

NGR=non-glucogenic ratio[acetate+butyrate]/[propionate].

[1] Efficiency of conversion of hexose into VFA energy.

the rumen liquor with forage diets reflect broadly the relative rates of production of VFAs beneficial to the ruminant animal. The data on ruminal ammonia concentration were within the range reported in the literature. Table 2 shows that with the R1 diet ammonia level of 2.63 mM was reached which is equal to 3.68 mg NH_3-N/100 ml rumen fluid. Satter and Roffler (1981) stated that the critical concentration of NH_3-N for maintaining maximum rumen microbial growth is about 2 mg NH_3-N/100 ml rumen fluid and a normal fluctuation about a mean concentration of 5 mg NH_3-N/100 ml rumen fluid, i.e. between 3 and 8 mg NH_3-N/100 ml due to feeding regime would support normal microbial growth. Considerable published work has demonstrated the need for rumen fluid ammonia concentrations to be held higher than 5 mg NH_3-N/100 ml, i.e. 15-20 mg NH_3-N/100 ml, to support maximum microbial production in the rumen (Leng and Nolan, 1984). The actual levels of ammonia required for growth and activity of rumen micro-organisms depend on a wide variety of factors including diet and feeding regime. With rations R2, R3 and R4, ammonia concentrations increased to 5.24 to 7.07, mM or 7.34 to 9.90 mg NH_3-N/100 ml rumen fluid, which met the universally accepted requirement for maximum microbial growth mentioned earlier. For comparison, Putra (1999) reported for late pregnant Bali cows fed the same type of diets as in the present study, ruminal NH_3 values ranging from 2.54 (grass-legume ration) to 6.62 mM (concentrate supplemented ration). Values for local tropical sheep receiving various concentrate supple- mented rations ranged from 7.21 to 11.04 mM (Zain, 1999).

If the same diets with CP contents ranging between 12.05 to 16.9% and TDN 58 to 66% were fed to temperate cattle, ammonia values could be calculated using equation NH_3-N (mg/100 ml)=38.73-3.04% CP+0.171% CP^2-0.49% TDN+0.0024% TDN^2; r^2=0.92 of Satter and Roffler (1981), arriving at values for R1, R2, R3 and R4 of 6.54, 17.76, 13.79 and 13.79 mg NH_3-N/100 ml, respectively. At the given combination of dietary protein and energy of the present study, Bali cattle maintained lower concentrations of ruminal ammonia than temperate cattle, indicating that Bali cattle would need more dietary crude protein level to make ammonia to accumulate to levels comparable to those of temperate breeds. Thus, Bali cattle would be more likely to benefit from NPN supplementation in the diet based on tropical forage with its low CP content.

Examining the results on bacterial counts and production of microbial protein where all values increased with the improved diets, excess ammonia (above 5 mg NH_3-N/100 ml) in Bali cattle would appear to have no adverse effect on rumen micro-organisms. It should be remembered however, that the present ammonia data were from rumen fluid samples taken 3 h post feeding, thus during peak fermentation. Due to normal fluctuation associated with twice-daily feeding practice, a peak concentration of 6 to 7 mM (or 9 to 10 mg NH_3-N/100 ml) would produce a mean concentration of around 5 mg NH_3-N/100 ml, a situation where microbial growth is maximally maintained. The data on bacterial count and production of microbial protein of R2, R3 and R4 (table 2) support this line of thinking. These data also indicate that there was no excess ruminal ammonia accumulation with the improved diets, due to ammonia uptake by rumen micro-organisms. Hence, although little ammonia would pass through the rumen wall and enter the peripheral circulation it would cause no implication on energy-linked transport processes of tissue cells.

R2 diet contained *Hibiscus* leaves which have a defaunating effect. Although not significantly different, protozoa count with the R2 diet decreased as compared to diet treatment R1. This defaunating advantage was not shown by diet treatments R3 and R4 even though both contained *Hibiscus* leaves but at a lower level (75% of R2). Protozoal count with R3 and R4 diets increased even more than twice that with R1 or R2. It seemed that the addition of concentrate may had counteracted the defaunating effect of *Hibiscus* leaves. Due to its higher digestibility, protozoa would still have a beneficial influence on protein and easily digestible carbohydrate supply to the small intestine of the ruminant host animal.

The results of body composition are presented in table 3. The data show that Bali cows seem to lose less weight and could maintain adequate body condition during lactation, which may be ascribed to their low level of milk production. Average daily gain (ADG) was negative, -7.4 to -71.4 g/d except for the R3 group (+93.75 g/d), equaling daily values around -0.003% to +0.037% of BW, which emphasized insignificant change in body weight. The time-course change of bi-weekly average body weight of the treatment groups of cows during the 16 wk experiment are depicted in figure 3.

Mean body protein retention was also negative for R1 and R2, but was positive with the R3 and R4 treatments; the values ranged from -2.11 (R2) to +9.00 (R3) g/d. Rprotein data of body can be translated into gain of body meat arriving at values of -1.40, -14.07, +60.07 and +2.26 g/d for R1, R2, R3 and R4, respectively. The discrepancy with the actual measured ADG data is small and may probably be attributed to differences in method of determination. Retained body fat was positive for all groups of cows except for the R2 group; the values were 24.81 g/d for R3 and -2.81 for R2. Retained body energy went parallel to retained fat; mean values were 1.127 MJ/d for R3 and -0.015

Table 3. Body composition (mean values) of first lactation Bali cows during the first 16 weeks of the lactation period

Body components	R1	R2	R3	R4	Statistical sign. test	SEM
Initial body mass (kg)	266.67	258.0	258.5	272.0	NS	12.06
Final body mass (kg)	265.83	250.0	269.0	265.67	NS	14.37
Initial body water (% BW)	48.72	49.17	48.96	48.49	NS	0.41
Final body water (% BW)	48.61	49.14	48.51	48.47	NS	0.34
Retained body fat (g/d)	1.12	-2.81	24.81	0.69	NS	14.35
Retained body protein (g/d)	-0.21	-2.11	9.01	0.34	NS	6.39
Retained body energy (MJ/d)	0.038	-0.015	1.127	0.033	NS	0.66
Average daily gain=ADG (g/d)	-7.4	-71.4	93.75	-55.1	NS	39.69

R1=elephant grass+*Gliricidia sepium* legume diet; R2=R1+*Hibiscus tilliacius* leaves; R3=R2+concentrate; R4=R3+Zn di-acetate (see text for details).
Values in a row with differing letter superscripts differ significantly at p level indicated.
SEM=standard error of the means due to feeding treatment.

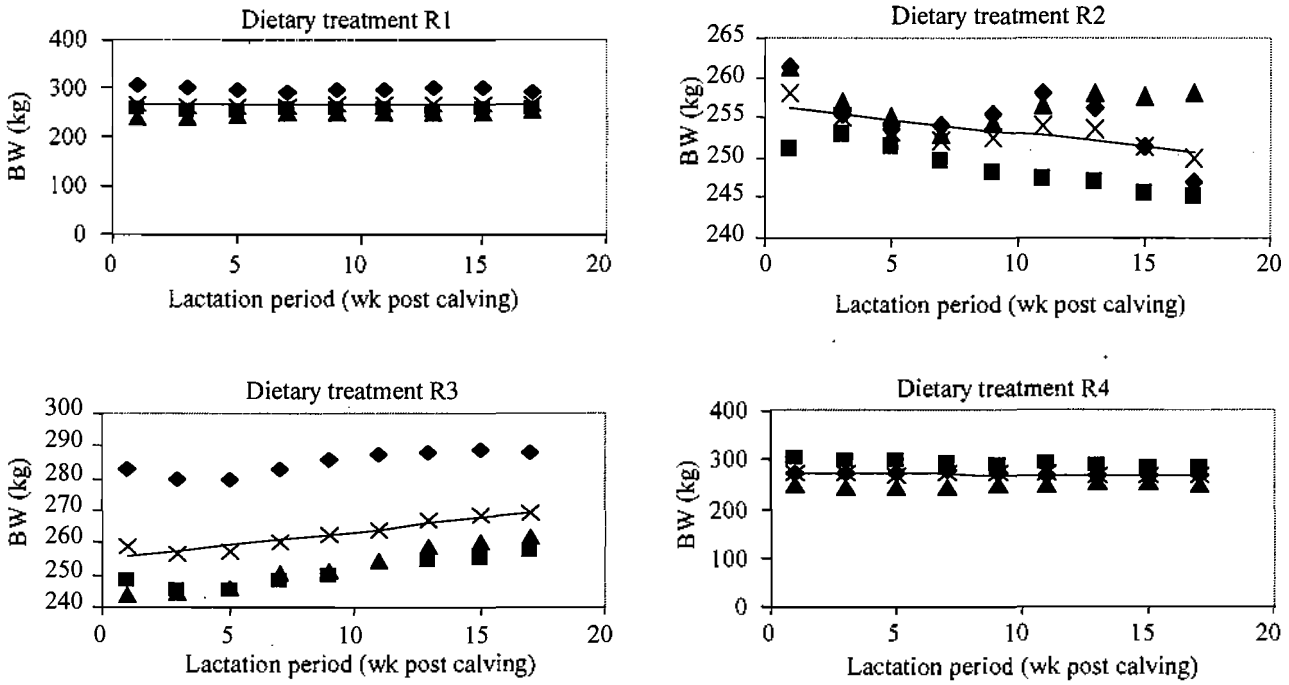

Figure 3. Scatter diagram of body weight changes during 17 week lactation period of first lactation Bali cows affected by dietary treatments R1, R2, R3 and R4 [three cows plus mean values (X) for each treatment group]

MJ/d for R2.

The results on milk production and composition are presented in table 4, and the daily milk yield measured at weekly intervals during the 16 wk observation period for the 4 dietary treatment groups are shown in figure 4. Milk yield increased with the improved diets ($p<0.01$), but even with ration R4, average daily milk yield was only 2.1 kg per cow which was about twice the average yield with the R1 diet. The composition of milk did not change significantly except for lactose ($p<0.01$) which had values for R1, R2, R3 and R4 of 5.04, 5.18, 4.92 and 4.86%, respectively. Total milk-DM, -protein, -fat, -lactose and -energy outputs increased significantly ($p<0.01$) however, and these were attributed to the increased yield. The data on milk reveal that protein contents were 4.93, 5.18, 4.97 and 5.08% for R1, R2, R3 and R4, respectively. Values in the same order for milk fat were 7.79, 7.98, 8.45 and 8.18%. Milk energy ranged from 4.87 to 5.38 MJ/kg. Milk fat and protein in the Bali breed of cattle is relatively high in comparison to other breeds. The weekly average daily milk production shown in figure 4 demonstrated that with the improved diets, the relatively high milk yields

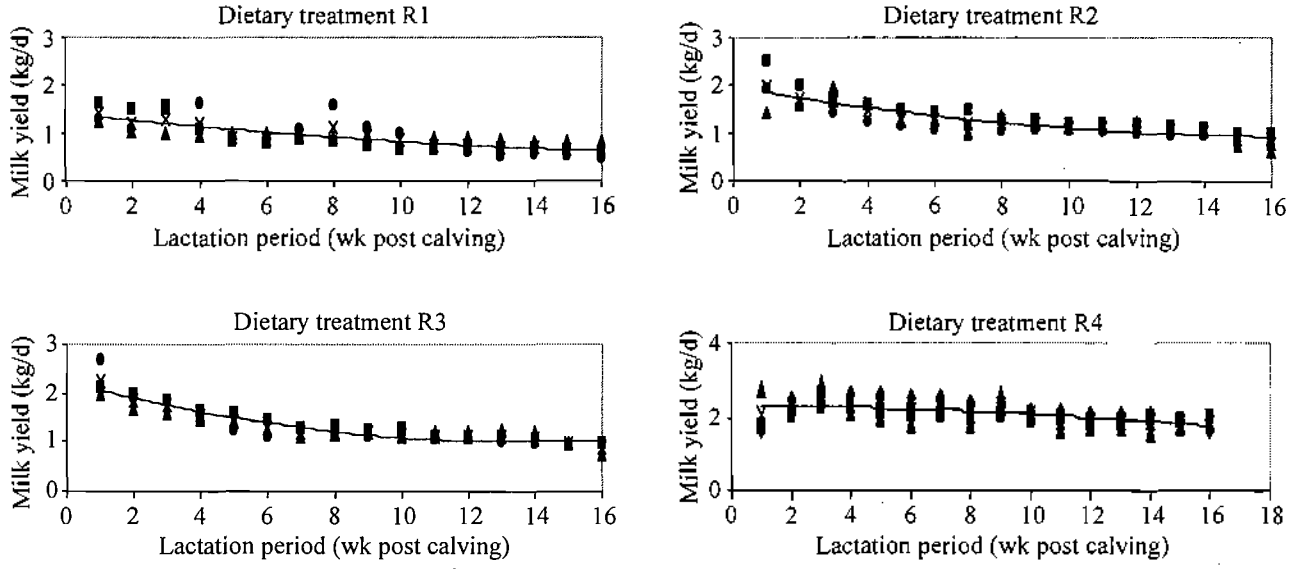

Figure 4. Scatter diagram of weekly recorded daily milk yield of first lactation Bali cows during 16 week lactation period on diets R1, R2, R3 and R4 [three cows plus mean values (X) for each treatment group]

were sustained much longer throughout the experimental period; especially the group on the R4 diet maintained a yield of 2 kg/d at week 16, slightly lower than the peak yield of 2.5 kg/d at wk 3 to 4. Milk of Bali cows contains relatively higher fat and protein compared to milk of Etawah crossbreed goats (Astuti et al., 2000).

Catabolized protein was determined as urinary N times 6.25, resulting in a mean value of 426.85 g/d for R1 and 1.64 to 1.74 times greater for the other treatment groups. Together with Rprotein of body tissues, milk protein is part of DP which is secreted as a milk component; therefore the amount of catabolized protein can be calculated as the difference between DP and the total Rprotein (in body tissues and milk). The values of catabolized protein calculated by both methods show close agreement. Energy equivalents of the catabolized protein appearing as N-waste products in urine were 2.105, 3.617, 3.427 and 3.503 MJ/d for R1, R2, R3 and R4, respectively or 0.034 MJ/kgW$^{0.75}$.d for R1 and 0.054 to 0.059 MJ/kgW$^{0.75}$.d for the other treatment groups. From these figures, ME values could be derived and mean values were found to be 0.722, 0.831, 0.781 and 0.870 MJ/kgW$^{0.75}$ per day for R1, R2, R3 and R4, respectively. The ratio ME/DE ranged from 82 to 84%. When ME values (MJ/d) were related to RE values (MJ/d), an equation was found: RE_{total}= 12.79-0.373 ME; (r=0.73) leading to an estimate of the daily ME for maintenance (ME_m) requirement for lactating Bali cows of 0.53 MJ/kgW$^{0.75}$. Requirement of ME to support the production of milk (Q_{mp}) ranging from 0.5 to 3.0 kg/d, follows the equation: Q_{mp} (kg/d)=-2.48+4.31 ME (MJ/kg$^{0.75}$.d), r=0.6; or if related to [ME - $RE_{body\ tissue}$](MJ/d): Q_{mp} (kg/d)=-3.4+ 0.08 [ME-$RE_{body\ tissue}$], r=0.94. Thus, in the present study ME intakes were 1.31, 1.51, 1.42 and 1.58 ME_m requirement for R1, R2, R3 and R4, respectively. Parallel to ME intakes, HP in lactating cows were elevated reaching 2.44 (R1) to 2.64 times BMR (R2) which demonstrated that lactation requires a high demand of body metabolism.

To calculate protein intake for maintenance (IP_m), an equation was found to relate RP_{total} to IP, i.e. RP_{total}=-47.45+0.12 IP; (r=0.74, n=9). Calculation showed that RPm=6.19 g/kgBW$^{0.75}$.d. These maintenance requirements are slightly higher for ME_m (0.50 MJ/kg$^{0.75}$.d) and lower than IP_m (10.8 g/kg$^{0.75}$.d) values found for first lactation Etawah crosbreed does (Astuti et al., 2000). To calculate protein requirement for lactation, the equation Q_{nl}=[(Q_{mp})(% protein in milk)(I_{mp})]/100 is used (Moen, 1973), where Q_{nl} is g protein required for lactation, Q_{mp} is quantity of daily milk yield, protein content of Bali cow's milk=5.04% (av. calculated from table 4) and I_{mp} is metabolic increment for milk production, i.e. ME_{lact}/ME_m where average value=1.46.

Summarizing the data on energy and protein metabolism, and milk yield of first lactation Bali cows: (1) Data on rumen fermentation products indicate that grass-legume diets are inadequate to supply protein, and concentrate supplementation is warranted; (2) Supplementation with concentrate to a level of 25% DM, and in addition, also Zn diacetate would improve ruminal digestion, milk yield and sustainability of yield for more than 16 weeks post calving; (3) ME_m requirement was 0.53 MJ/kg$^{0.75}$.d, while IP_m requirement was 6.19 g/kgBW$^{0.75}$.d. The

Table 4. Mean values of metabolic parameters, and milk yield and components of first lactation Bali cows during the first 16 weeks of lactation period

Nutritional parameters	Ration R1	Ration R2	Ration R3	Ration R4	Statistical sign. test	SEM
ME intake (MJ/kgW$^{0.75}$.d)	0.722^{a}	0.830^{a}	0.781^{a}	0.870^{a}	NS	0.029
ME/DE (%)	83.6^{a}	81.7^{b}	81.7^{b}	83.7^{a}	p<0.01	0.004
HP (MJ/kgW$^{0.75}$.d)	0.716^{a}	0.771^{a}	0.690^{a}	0.741^{a}	NS	0.029
Catabolized protein (g/d)	426.85^{a}	744.77^{b}	701.22^{b}	711.52^{b}	p<0.05	15.15
UE (MJ/ kgW$^{0.75}$.d)	0.034^{a}	0.059^{b}	0.054^{ab}	0.054^{ab}	p<0.05	0.001
(% GE)	2.32^{a}	3.55^{b}	3.55^{b}	3.52^{b}	p<0.01	0.035
RE in body+milk (MJ/W$^{0.75}$.d)	0.056^{a}	0.060^{a}	0.090^{ab}	0.129^{ab}	p<0.01	0.010
Rprotein in body+milk (g/d)*	45.14^{a}	63.22^{a}	74.1^{b}	106.08^{c}	p<0.01	7.72
Milk yield (kg/d)*	0.92^{a}	1.26^{b}	1.31^{b}	2.08^{c}	p<0.01	0.063
-Milk DM (g/d)*	169.52^{a}	232.39^{b}	252.90^{b}	393.01^{c}	p<0.01	12.69
-Milk protein (g/d)*	45.35^{a}	65.33^{b}	65.09^{b}	105.74^{c}	p<0.01	2.86
-Milk fat (g/d)*	71.69^{a}	94.19^{b}	110.75^{b}	170.22^{c}	p<0.01	6.44
-Milk lactose (g/d)*	46.36^{a}	65.13^{b}	64.59^{b}	101.34^{c}	p<0.01	3.43
-Milk energy (MJ/d)*	4.48^{a}	6.43^{a}	7.05^{a}	10.86^{b}	p<0.05	0.69
Milk energy (MJ/kg)	4.87^{a}	5.10^{a}	5.38^{a}	5.22^{a}	NS	0.38
En. eff. for milk production (%)	6.88^{a}	7.66^{a}	9.16^{a}	14.79^{b}	p<0.01	0.70
Eff. prot. util. for milk prod. (%)	9.81^{b}	8.40^{a}	8.77^{a}	13.33^{b}	p<0.05	2.28

R1=elephant grass+*Gliricidia sepium* legume diet; R2=R1+*Hibiscus tilliacius* leaves; R3=R2+concentrate; R4=R3+Zn diacetate (see text for details).

Values in a row differing letter superscripts differ significantly at p level indicated.

SEM=standard error of the means due to feeding treatment. DM=dry matter; DE=digestible energy; UE=urinary energy; ME=metabolizable energy; HP=heat production; RE=retained energy in body tissues and milk; RProtein= retained protein; En eff.=energetic efficiency; Eff. prot. util.=efficiency of protein utilization.

* Calculated from data during 16 week experiment.

equation $Q_{nl}=[(Q_{mp})(\%\text{ protein in milk})(I_{mp})]/100$ may be used to calculate protein requirement (Q_{nl}) for milk production; (4) Milk protein content of the lactating Bali cow ranges from 4.93 to 5.18% with average value of 5.04%, milk fat averages 7.98%, milk lactose 5.0% and milk energy was 5.14 MJ/kg. Milk fat and protein of Bali cattle is relatively high in comparison to other cattle breeds.

In conclusion, the present investigation with first lactation Bali cows reveals that the lactation potential of the Bali cow of the humid tropics still allows elevation of milk production by nutritional manipulation, but the underlying mechanisms implicating utilization of nutrients for milk production and associated processes should be examined. The mechanism responsible for mammary secretory activity for milk production should be clarified. Such studies are important considering the peculiar milk composit- ion, e.g. high milk fat and protein contents, of this breed of cattle.

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