Mammary Performance of First Lactation Bali Cows (*Bibos banteng*) Fed Grass-Legume Based Diets in Relation to the Role of Glucose[a]

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ABSTRACT : A study of mammary function in relation to glucose metabolism of first lactation Bali cows on grass-legume diets was carried out using 12 primiparous cows (initial BW 263.79±21.66 kg) for 16 weeks starting immediately post calving. The animals were randomly allocated into 4 dietary treatment groups R1, R2, R3 and R4, receiving from the last 2 months of pregnancy onwards, rations based on a mixture of locally available grass and legume feed *ad libitum*. On a DM basis R1 contained 70% elephant grass (PP, *Penicetum purpureum*) plus 30% *Gliricidia sepia* leaves (GS), R2 was 30% PP plus 25% GS supplemented with 55% *Hibiscus tilliacius* leaves (HT, defaunating effect), R3 and R4 were 22.5% PP+41.25% GS+11.25% HT+25% concentrate, with R4 supplemented with zinc-diacetate. 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 weights were monitored, an energy and protein balance trial conducted, overall glucose kinetics parameters assessed, mammary blood flow (MBF) and metabolite arteriovenous differences (ΔAVs) measured to get uptake data and mammary performance relationships. Parameters of glucose kinetics at peak lactation or during dry condition were not affected by ration quality. Glucose pool size, space of distribution and flux increased by 61.77, 62.26 and 82.08%, respectively, during lactation compared to the dry period. Mean glucose flux of lactating Bali cows was 5.52 mg/min.kgBW$^{0.807}$ which resembles the range of values of temperate dairy cows. Calculation showed that glucose requirements for maintenance, milk lactose and fat-glycerol synthesis, and the formation of NADPH reached 461.69 g for a yield of 1 kg/d or equal to 320.62 mg/min, which was less than the average glucose flux of lactating Bali cows of 481.35 mg/min. Mammary blood flow (MBF) values ranged from 56 to 83 l/h for the different treatments and the ratio MBF per kg milk produced improved from av. 1540 l/kg for R1 to av. 967 l/kg for R4 treated cows. Mammary glucose uptake ranged from 6.27 to 12.03 g/h or 120 to 140 g/kg milk. Glucose uptake was mass-wise 2 to 4 times the amount secreted as lactose, which indicated values less than the calculated mammary glucose needs and that little lactose was synthesized. The excess glucose taken-up was used for other metabolic processes. Linear relationships between metabolite Δ AVs and arterial blood plasma concentration [A] showed that in Bali cows triglycerides (TG), phenylalanine (Phe) and tyrosine (Tyr) have high coefficients of determination, i.e. 0.77, 0.81 and 0.69, respectively. For glucose, the relationship is quadratic with an R^2 value of 0.49. It was concluded that lactose synthesis was inadequate, which led to a speculation that milk yield could be improved by increased lactose synthesis. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 5 : 615-623*)

Key Words : Bali Cattle, *Bibos banteng*, Lactation, Mammary Performance, Metabolite Uptake, Glucose

INTRODUCTION

Bali cattle are an indigenous breed that is found mainly in lowland areas on Indonesian islands near the equator. They are known to have good fertility but are poor milk producers with small udders and lactation length varying from 6 to 10 months producing 0.9~2.8 kg per day. This situation leads to the slow growth of calves and ultimately to high mortality during the pre-weaning period (Soehadji, 1991) especially for calves born in the dry season under extensive management (Liwa, 1992; Wirdahayati, 1998).

Little basic research has been conducted on the biology of this cattle breed, especially with regard to lactation potential. It was recently reported that the milk production response of lactating Bali cows to improved diets based on a mixture of grass and legumes, with or without concentrate supplements, increased with quality of diet (Sukarini et al., 2000). It was desired that the mechanism responsible for mammary secretory activity for milk production should be disclosed. Thus additional to that report, the present publication deals with the performance of the lactating mammary gland in relation to general glucose metabolism and its associated regulatory role in mammary metabolism connected to some related energetic metabolites and amino acids.

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Glucose is an important intermediate in general metabolism and plays a particular role in mammary metabolism (Chaiyabutr et al., 2000). The role of glucose is evaluated based on: (i) the extent of glucose utilization by the secretory organ; (ii) the biosynthesis of lactose and (iii) the role of glucose in regulating milk secretion (Mepham, 1993). Lactose, the major osmo-regulator of milk, is synthesized in mammary tissue from glucose and is a major energetic substrate in lactating animals. Milk composition and mammary energetics are intimately related, e,g. arteriovenous difference (ΔAV) of glucose is relatively independent of blood concentration, but ΔAVs of critical amino acids and many energetic nutrients are a function of their concentrations in arterial blood (Miller et al., 1991; Cant et al., 1993b). To investigate mammary function of the Bali cow, the relationship between mammary blood flow (MBF) and milk yield, mammary ΔAVs and uptake of several metabolites were measured. Special attention was paid to glucose where its supply is secured by feeds rich in soluble carbohydrates or glucogenic precursors. Abundant supply of glucose to the mammary gland would assure uptake for increased milk lactose synthesis and in turn, due to lactose being the major osmo regulator of milk, would augment milk yield. The practical implication derived from this study would be feeding Bali cattle with fodder silage or supplementation with concentrate and minerals under the three strata feeding system (Nitis et al., 1990) ensuring adequate supply of glucose precursors and activators for milk lactose synthesis.

MATERIALS AND METHODS

Animals and feeding

Details of the experiment have been reported by Sukarini et al. (2000). Briefly, twelve primiparous 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 was of a randomized block 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*. The dietary treatments began 1 to 2 months prior to calving. The diets were R1 consisting of 70% elephant grass (PP, *Penicetum purpureum*) plus 30% *Gliricidia sepia* leaves (GS), R2 was 30% PP plus 25% GS supplemented with 55% *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 supplemented with zinc-diacetate. 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. Body weight and milk production were continuously monitored, where morning milking was done before feeding, body composition estimated at the start and at the end of the experimental period, and a 7 day energy- and N-balance trial performed on the 3rd to 4th week of the experiment. Blood samples were withdrawn post-feeding, co-incident with post-milking on the morning of the last day of the balance trial, under minor physical restraint. The samples were drawn from either the left or right caudal superficial epigastric vein and coccygeal artery or vein of each cow for the purpose of measuring blood metabolites levels and mammary arterial-venous differences (Δ AV). Emery et al. (1965) validated the use of coccygeal vein samples as indices of arterial blood metabolite concentrations. (Blood sampling protocols are described further under: Measurement of mammary function). Within one week after termination of the balance trial, 2 cows of each treatment group were used to obtain data on glucose kinetics. The glucose kinetics trial was repeated when the same cows were dry, but still receiving the treatment rations, about 30 weeks post partum. By the end of the balance trial, rumen fluid from each cow was taken by way of a stomach tube for measuring *in vitro* ruminal fermentation characteristics.

Glucose kinetics trial

Glucose kinetics studies were carried out using the pulse labeling technique of glucose-2-^{3}H as previously described (Sastradipradja et al., 1976; Sastradipradja, 1992). Each cow received intravenously a dose of 230 μCi and serial blood samples were withdrawn from the jugular vein via implanted catheters at 0 min., and at every 20 minutes post-injection up to 120 minutes. Blood samples (10 ml) were immediately transferred to a centrifuge tube containing 5 mg of sodium fluoride and 2 drops of heparin (10 IU/ml) and placed on ice to chill. The blood samples were then centrifuged to separate the plasma from the blood corpuscular elements. The plasma was transferred into capped plastic tubes and stored frozen at -20°C until assay for glucose concentration and radioactivity. The glucose-penta-acetate (GPA) derivative method (Jones, 1965) was used for measuring plasma tritiated glucose specific activity (SA) for calculation of glucose pool, flux and space of distribution.

Measurement of mammary function

To calculate uptake of blood metabolites by the mammary gland from ΔAVs, the Fick principle (tissue uptake=ΔAV×blood flow) was used. Estimation of mammary blood flow (MBF) was based on this

principle and the selected indicator compound that is stoichiometrically transferred from blood into milk is phenylalanine (Phe) plus tyrosine (Tyr) combined (Cant et al., 1993a) and MBF (liters per hour) was calculated as follows:

$$MBF=[(FY_B \times 0.965)+FY_F]/FY_{A\text{-}V}$$

where FY_B=Phe+Tyr output bound in milk protein (moles per hour), FY_F=free milk Phe and Tyr (moles per hour), $FY_{A\text{-}V}$=(Phe+Tyr) ΔAV (moles per liter), 0.965=correction factor assuming blood-borne proteins constituting 3.5% of the total milk protein.

Values for FY_B were estimated from yield of casein and whey protein over 12 h period. FYB values were calculated from reversed-phase HPLC determinations. Similarly, free FYF values used reversed-phase HPLC analysis of amino-acid content in 1 ml of milk that was deproteinized with 100 μl of 50% sulfosalicylic acid.

In order to associate the collected milk with blood samples and assuming that transit times of glucose and acetate through the gland are the same as for the goat, 2 and 6 h (Cant et al., 1993b), blood was sampled three times, i.e. 2 h after the last feeding for glucose determination, at 4 h thereafter for tryglyceride (TG) and acetate (Ac) determination, and 3 h after the last sampling for amino-acid (AA) analysis. The cows were then immediately milked after injection of oxytocin, the yield recorded and aliquots were taken for amino-acid analysis of casein and milk whey. Metabolite uptake by the mammary gland entailed the use of the Fick principle according to equation (Cant et al., 1993b):

$$v=\text{uptake}=\Delta AV \times MBF$$

where v=steady state velocity of transport (g or moles per hour), ΔAV=arteriovenous concentration difference of metabolite (befitting g or moles per liter) for glucose, TG, Ac and some selected AAs.

Statistical analysis

The significance of difference between means was compared using the Duncan Multiple Range Test after ANOVA (Steel and Torrie, 1986). Equation of mathematical models relating measured variables i.e. between v of a particular metabolite vs. its arterial concentration A, and between ΔAV vs. A, was obtained by regression analysis using Lotus 123 program and Minitab.

RESULTS AND DISCUSSION

Results on digestibilities of ration components, ruminal fermentation data, energy and protein balances, body composition and milk production have been reported earlier (Sukarini et al., 2000). Briefly, results indicated that milk production of Bali cows could be enhanced by supplementation of legumes (*Gliricidia*) above traditional grass rations and by using browsing leaves having a defaunating effect (*Hibiscus*) causing reduction of protozoal but increase rumen bacterial population, yielding an average increase from 0.92 (R1) to 1.26 kg milk/d (R2). Further improvement by concentrate supplementation (at a level of 25% DM) allowed an increase in ruminal total and partial volatile fatty acids (VFAs), especially propionate, which improved milk yield. Addition of zinc-diacetate to the concentrate supplemented grass-legume diet improved ruminal fermentation, ME, RE in body tissues and energy in milk and retained protein enabling milk yield to increase to 2.08 kg/d (R4) and being sustained longer at that level of production.

Glucose kinetics

Parameters of glucose kinetics of Bali cows either at peak lactation or during dry condition were not affected by ration quality as shown in table 1. In general however, improved feeding resulted in a tendency of increasing glucose pool size, space of distribution and flux. The group of animals undergoing weight loss during lactation (group R2 and R4), were due to lipolytic capacity of adipose tissue. The animals may have to replenish fat resources during the subsequent dry period (Baldwin and Kim, 1993) and such may need glucose as a source of NADPH for long-chain fatty acid synthesis in body adipose tissues, hence the high glucose flux values of dry cows of both treatment groups in question. Due to the fact that no effects on glucose flux were obtained by improved feeding during lactation as well as under dry condition, it would be reasonable if all the data of lactating animals are analysed together (n=8) and compared with the similarly treated data of the dry cows. The results are shown in table 2. Except for plasma glucose concentration, all other glucose kinetics parameters were higher for lactating cows than when they were non lactating, i.e. pool size, space of distribution and flux increased by 61.77, 62.26 and 82.08%, respectively. The glucose flux data demonstrated that the mean values for lactating cows was 481.35 mg/min against 264.36 mg/min for dry cows or equivalent to 5.52 versus 2.99 mg/min.kgBW$^{0.807}$. Glucose flux of the lactating Bali cow with an average BW of 263.7 kg resembled the flux value of lactating cows of temperate climates reported by Anand (1969) measuring 793 mg/min (BW 472 kg), equivalent to 5.50 mg/min.kgBW$^{0.807}$. The average value for the dry Bali cow is slightly higher than the standard fasting value according to Ballard et al. (1969) as modified by Astuti et al. (2000) of 2.86 mg/min.kgBW$^{0.807}$. The discrepancy was caused by the fact that the dry Bali cows had access to feed. Thus

Table 1. Parameters of glucose kinetics of first lactation Bali cows during peak lactation and subsequent dry condition of the same cows fed grass-legume based diets

Glucose kinetics parameter	Ration treatment								Statistical test		
	R1		R2		R3		R4				
	Peak* (n=2)	Dry* (n=2)	Peak* (n=2)	Dry* (n=2)	Peak* (n=2)	Dry* (n=2)	Peak* (n=2)	Dry* (n=2)	Peak	Dry	SEM
Blood plasma concentration (mg/dl)	86.0	75.75	85.5	80.5	87.0	79.0	78.0	86.5	NS	NS	NS
Pool size (g/animal)	32.59	15.91	40.55	33.84	38.78	13.30	49.92	36.99	NS	NS	NS
Space of distribution (%BW)	14.72	8.17	19.18	15.96	16.67	6.22	24.58	15.95	NS	NS	NS
Flux (mg/min.animal)	355.76	209.40	852.89	347.97	403.73	136.02	313.03	364.07	NS	NS	NS
Flux (mg/min.kgBW$^{0.807}$)	4.12	2.4	9.93	3.90	4.51	1.48	3.54	4.18	NS	NS	NS
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)	3.55[a]		3.94[a]		4.71[a]		8.55[b]		p<0.05		0.69

* Mean values, peak=during condition of peak lactation, dry=during dry condition.

NS: non-significant among lactating as well as among dry cows.

** Milk yield and composition data (n=3 for each treatment group) from: Sukarini et al. (2000).

for the calculation of glucose maintenance requirement it is safe to take the standard value of 2.86 mg/min.kgBW$^{0.807}$. Glucose requirement for maintenance for the Bali cow would be 370.41 g/d.

Assuming the extent of synthesis of milk components of the Bali cow is similar to dairy cattle, for the synthesis of lactose, the amount of glucose needs would be 52.63 g/kg milk, taking into consideration that the average milk lactose content of Bali cows is 5.0% and assuming that for the synthesis of 1 mole of lactose (342 g), 2 moles of glucose (360 g) is needed. Glucose for TG-glycerol formation is 12% of the amount of fatty acids being synthesized (Boekholdt, 1976), thus the amount for the Bali cow milk with 7.97% average fat concentration would be 9.56 g glucose/kg milk. This estimated value is a maximal value considering milk fat also originates from fat in feed. For the synthesis of milk long-chain fatty acids, glucose requirements to provide the reducing power NADPH through the glucose pentose phosphate pathway were calculated, based on the assumption that for the synthesis of 1 kg milk fat, 49 moles of NADPH are needed - these were derived from 4.06 moles glucose to be equivalent to 730 g glucose. It was reported that the maximum glucose required for fatty acid synthesis is 73% of the fat quantity (Boekholdt, 1976). Summing up, the glucose requirement for the milk production of Bali cows with 5.0% lactose and 7.97% fat and with the assumption that 50% of the NADPH requirement will be supplied by glucose, was found to be 91.28 g/kg milk.

For a yield of 0.5, 1, 2 and 3 kg milk, adding maintenance glucose requirement, the total requirements were then 416.05, 461.69, 552.97 and 644.25 g glucose, respectively. Given the requirement of 461.69 g glucose for cows producing 1 kg milk/d, a flux to the mammary gland of 320.62 mg/min is required. In comparison, the average body glucose flux of 481.35 mg/min for the lactating Bali cow (table 2) is larger than the calculated requirement for milk production. Thus, the existing glucose flux should be adequate to support milk production around 2 kg/d.

MBF and nutrient uptake by the mammary gland

Quantitative assessment of nutrient utilization for milk production by the mammary gland in the present study relies on the use of the Fick principle to measure MBF and uptakes of nutrients/metabolites by the mammary gland. The data on these parameters are presented in table 3. MBF values ranged from 56 to 83 l/h for the different treatments.

Improvement of feed quality increased MBF with a significant increment of 42% (R4) above the basal grass/legume diet (R1). The ratio between MBF to support the production of 1 kg milk improves from 1540 l/kg for ration R1 to 967 l/kg milk for ration R4, which means that a more efficient synthesis and secretory process is reached with improved diets. The mean value for the R4 treated cows, 967 l/kg milk is close to the value of 724 to 913 l/kg milk reported by Cant et al. (1993b) for dairy cows yielding milk around 25 kg/d and fed fat supplemented rations. The ratio with the Bali cow is even more disadvantageous if compared to the values of dairy animals of around

Table 2. Glucose kinetics of a group of Bali cows during peak first lactation and subsequent dry condition (mean ± SD)

Glucose kinetics parameter	Lactational condition		Statistical test
	Dry condition	Peak lactation	p value
	(n=8)	(n=8)	
Blood plasma concentration (mg/dl)	80.44 ±2.60	84.12 ±2.10	NS
Pool size (g/animal)	25.01 ±6.30	40.46 ±4.70	p=0.03
Space of distribution (%BW)	11.58 ±2.70	18.79 ±2.10	p=0.04
Flux (mg/min.animal)	264.36 ±43.0	481.35 ±103.0	p=0.06
Flux (mg/min .kgBW$^{0.807}$)	2.99 ±0.48	5.52 ±1.20	p=0.07

NS: non significant at $p<0.05$.

500 l/kg milk as reported by Linzell (1974), Bickerstaffe et al. (1974), Waghorn and Baldwin (1984), Davis and Collier (1985) and Guinard et al. (1994). The observation on the Bali cow however, may indicate that there is potential for this breed of cattle to produce more milk, either by improved feeding management or by other metabolic modulations. It should be remembered however, that evaluation of milk production potential by comparing ratios of MBF per kg milk between the Bali cow and the standard dairy animal is not a good way to exercise considering that milk compositions quantitatively differ widely between the breeds.

The main substrates taken up by the mammary gland are glucose, TG, Ac and AAs as precursors of milk lactose, fat and protein. Nutrient uptake by the mammary gland e.g. for glucose and TG increased with improved feeding. The same observation holds true for Phe and Tyr uptakes. Ac uptake on the other hand was highest with R1, decreased somewhat with R2 and R3, but increased again with treatment R4. The high Ac uptake value of R1 was attributed to ration composition consisting of 100% forage, while ration R3 was concentrate supplemented diet causing lowered Ac production. The high Ac uptake with R4 diet was due to the presence of adequate Zn causing improvement of ruminal bacterial growth and mammary secretory cell development.

Mammary ΔAVs for glucose in the present experiments ranged from 8 to 20 mg/100 ml blood, and were within the range of values reported by Linzell (1974) in lactating goats of 16 mg/100 ml blood with a ratio MBF over milk yield of 500 l/kg. With this ratio, a mean glucose uptake of 8.0 g/100 ml milk secreted were obtained which means that almost double the amount being secreted as lactose in 100 ml milk with 4.5% concentration. Glucose uptake by the Bali cow's mammary glands ranged from 6.27 to 12.03 g/h or 120 to 140 g/kg milk, which are almost twice the values for goats and dairy cows, 57 mg/ml (dairy cow) as reported by Waghorn and Baldwin (1984), 60 g/kg (dairy cows) by Guinard et al. (1994), 80 g/kg (dairy cow) by Cant et al. (1993b) and 70 to 80 g/kg (goats) found by Kronfeld (1982). Glucose uptake by Bali cow's mammary gland was mass-wise 2 to 4 times the amount secreted as lactose, while Annison et al. (1974) stated that glucose as a major metabolite for milk synthesis, would have a glucose rate of uptake by mammary cells reaching approximately twice the output of lactose. This observation re the Bali cow suggested that glucose was not only used for milk lactose synthesis, but is also required for NADPH formation related to long-chain fatty acid synthesis from Ac considering the higher milk fat content (±8%) of Bali cow's milk. Glucose would also contribute to energy production (ATP) required by the functioning mammary gland to support anabolic reactions including milk protein

Table 3. Metabolic performance of the mammary gland of first lactation Bali cows on grass-legume based diets

Metabolic parameter	R1	R2	R3	R4	Statistical sign. test	SEM
MBF rate (1/h)	59.01^{ab}	76.68^{bc}	56.13^{a}	83.80^{c}	p<0.01	3.471
MBF per kg milk (1/kg milk)	1540.27^{b}	1463.71^{b}	1025.77^{b}	967.64^{a}	p<0.05	58.929
ΔAV glucose (mg/100 ml)	14.00^{ab}	8.00^{a}	20.33^{b}	14.33^{ab}	p<0.05	1.633
ΔAV triglyceride (mg/100 ml)	10.00^{a}	12.33^{b}	15.33^{b}	15.66^{b}	p<0.05	1.151
ΔAV acetate (mM)	14.46^{c}	2.02^{a}	10.32^{b}	13.82^{bc}	p<0.05	1.936
ΔAV phenylalanine (nM)	30.99^{a}	55.05^{b}	57.82^{b}	24.52^{a}	p<0.01	1.693
ΔAV tyrosine (nM)	18.62^{a}	33.09^{b}	96.57^{c}	23.29^{b}	p<0.01	2.440
Glucose uptake (g/h)	8.28^{ab}	6.27^{a}	11.23^{bc}	12.03^{c}	p<0.05	1.021
Triglyceride uptake (g/h)	5.91^{a}	9.64^{bc}	8.57^{b}	12.98^{c}	p<0.05	1.112
Acetate uptake (moles/h)	0.85^{b}	0.16^{a}	0.58^{b}	1.13^{b}	p<0.01	0.119
Phe uptake (nmoles/h)	1830.60^{a}	4206.30^{b}	3239.00^{b}	1992.00^{a}	p<0.01	276.347

synthesis. Glucose may also be involved providing carbon skeleton for other milk components. It has been reported that lipoprotein lipase in mammary capillaries hydrolyzes plasma TG, and the fatty acids released are taken up by mammary cells (West et al., 1972). Thus ΔAV of TG represents TG hydrolysis allowing a flow of fatty acids to be taken up by mammary cells sparing the use of Ac for long-chain fatty acid synthesis. Therefore, the fate of the high Ac uptake by mammary cells would most likely be for ATP formation where much is needed for milk protein synthesis (Preston and Leng, 1987). This view is supported by the high protein content of Bali cow's milk.

In theory, metabolite uptake involving cell membrane protein binding would lead to functional relationship between the pattern of metabolite transport into cells with their concentrations in arterial blood (Cant et al., 1993b). These authors stated that uptake velocity is a function of nutrient concentration, level of modulator (inhibitor/activator) and the characteristics of the membrane protein systems following the Henri-Michaelis-Menten equation:

$$v=\text{uptake}=\Delta AV\times MBF=\frac{V_{maxapp}}{1+K_{sapp}/[A]}$$

where: v=nutrient uptake (moles/h), ΔAV=arterial-venous difference of nutrient/metabolite concentration (moles/l), MBF=mammary blood flow rate (l/h), [A]= arterial concentration of nutrient/metabolite (moles/l), V_{maxapp}=apparent maximum uptake of substrate (moles/h) and K_{sapp}=apparent transport affinity.

The Henri-Michaelis-Menten equation is based on biological mechanism (transport mechanisms) whereas the Fick principle is unable to estimate uptake by the organ from blood measurements. An increase in blood flow does not necessarily mean increased uptake and local controls of blood flow by pre-capillary vasoconstrictors will have their influence. Opening of capillary valves will cause protein transport together with other nutrients in blood reflecting maximal increase of the system. Thus, an increase of nutrient uptake will be reached. Therefore, assuming V_{maxapp} as function of true maximum uptake and MBF, the relation between ΔAV and arterial concentration will be apparent as shown by the following derivation.

$$\Delta AV\times MBF=f(V_{max}\times MBF)/\{1+K_{sapp}/[A]\} \text{ and}$$
$$\Delta AV=f(V_{max})/\{1+K_{sapp}/[A]\}$$

As a consequence, ΔAV and uptake values correlate with arterial concentrations. Cant et al. (1993a,b) reported that uptakes have lower correlations with their respective [A] as compared to [ΔAV] vs [A] relationships. Baldwin and Kim (1993) stated that over a normal physiological range of concentrations, nutrient uptakes by the udder are essentially linear, i.e. normal concentrations of many metabolites are at or below the Ks. In these cases blood concentrations of nutrients can be represented as the primary determinants of uptake. Calculations of these relationships in Bali cows are presented in table 4.

The coefficient of determination of some milk precursors for ΔAVs vs [A] relationships are generally high for dairy cattle on a high fat diet, although the coefficient for glucose is low (Cant et al., 1993b; Miller et al., 1991) leading to the conclusion that glucose concentration is not limiting milk synthesis and factors other than glucose concentration determine glucose uptake, e.g. availability

Table 4. Statistical coefficients for linear regression equations for milk production performance of first lactation Bali cows

		Intercept			Slope			
	n	Estimate	SD	p	Estimate	SD	P	r^2
[ΔAV] vs [A]								
Glucose (mg/dl vs. mg/dl)	12	2.21	13.68	0.875	0.24	0.273	0.40	0.07
Triglyceride (g/l vs. g/l)	10	0.068	0.013	0.00	0.184	0.36	0.00	0.77
Acetate (mmoles/l vs. mmoles/l)	12	8.795	5.733	0.156	0.065	0.256	0.806	0.006
Phenylalanine (nmoles/l vs. nmoles/l)	11	-11.956	8.858	0.21	0.597	0.097	0.00	0.81
Tyrosine (nmoles/l vs. nmoles/l)	12	-33.39	17.21	0.081	0.844	0.18	0.00	0.69
[v] vs [A]								
Glucose (mg/h vs. mg/dl)	12	-2.062	2.809	0.783	231.5	145.6	0.143	0.25
Triglyceride (g/h vs. g/l)	10	4.359	4.644	0.375	12.46	12.12	0.334	0.12
Acetate (mmoles/h vs. mmoles/l)	12	758.2	415.3	0.098	-3.21	18.54	0.866	0.003
Phenylalanine (nmoles/h vs.nmoles/l)	10	-701.8	950.9	0.482	39.63	10.82	0.006	0.63
Tyrosine (nmoles/h vs nmoles/l)	12	-1261.4	899.3	0.191	44.245	9.401	0.00	0.69

[ΔAV]: arteriovenous difference; [A]: metabolite concentration in arterial blood plasma.
[v]: metabolite uptake by mammary gland; r^2: coefficient of determination (linear equation).

of Ac and AA to the gland (Miller et al., 1991a). Calculations based on linear relationship between ⊿AV vs A show that with Bali cows only TG, Phe and Tyr has high determination coefficients, i.e. 0.77, 0.81 and 0.69, respectively, while for Ac: r^2=0.006 and glucose: r^2=0.07. The values for Ac and glucose are contrary to the findings by Cant et al. (1993b), where the respective values were 0.80 and 0.12. The different results may be attributed to the small replicates used in our present experiment. However, the relation of glucose uptake (y, mmoles/h) vs arterial concentration (x, mmoles/L) is quadratic (figure 1), i.e. $y=10.221x^2-50.973x+66.065$ with a coefficient of determination R^2=0.49 ($p<0.05$), which indicated that glucose uptake followed more closely the Henri-Michaelis-Menten mechanism and that glucose uptake was quite dependent of arterial concentration. In addition, as is depicted in figure 2, a quadratic relationship was found between ⊿AV glucose (y, mmoles/L) and ⊿AV Ac (x, mmoles/L), following the equation $y=-0.006x+0.136x+0.2126$; R^2=0.57 ($p<0.05$) which clearly indicates that Ac availability to the gland determines glucose uptake. Greater Ac availability increases rates of NADPH utilization for fatty acid synthesis and, as a result, NADP concentration increased limiting the rate of glucose-6-P dehydrogenase reaction which causes accumulation of glucose-6-P and in turn feeds back negatively on the hexokinase reaction (Baldwin and Kim, 1993).

Glucose uptake values ranged from 6.27 to 12.03 g/h (104.5 to 200.5 mg/min) for Bali cows producing 0.9 to 2.08 kg/d. Viewing the data on glucose flux of lactating Bali cows of 481.35 mg/min, there is indication that with all the rations tested, glucose supplies have been satisfied, but may indicate that glucose transport could still be improved. Prosser (1988) detected the existence of a specific transporter for glucose in mammary cell membranes of rats which indicates that glucose uptake is regulated by the transporter. This phenomenon is also evident from the observation with Bali cows where the coefficient of determination of the quadratic glucose [v] vs [A] relationship is adequate, R^2=0.49. In addition, considering the fact that lactose concentration (and production rate) in Bali cow's milk is not much higher than that of dairy cow's milk, while for milk-fat and protein contents Bali cow's milk is superior, it would be reasonable to conclude that the low milk yield of the Bali cow is attributed to low milk lactose synthesis.

TG uptake of Bali cows undergoing treatment R4 was 12.98 g/h, while for cows of the other treatment groups the values were lower (5.9 to 9.64 g/h). These values were in excess of milk fat indicating that part of the TG taken up by the mammary gland would be metabolized for other purposes. The pattern of Ac uptake is not clear, however, with treatment R4 a value of 1130 mmoles/h was obtained, which indicated that much of it would be used for catabolic mammary metabolism. The Phe uptake data was high (ration R2 produced the highest value of 4206.3 nmoles/h) which together with Tyr uptake data (Tyr culminated with ration R3, 5385.34 nmoles/h) indicated sufficient supply for milk protein synthesis. The demand for energy (TG) and protein by Bali calves for growth was satisfied even though volume-wise Bali cow's milk is low but has high nutrient contents.

Glucose as a major precursor of lactose would provide 85% of lactose carbon (Bickerstaffe et al., 1974). From the present experiment it was calculated that milk lactose was only 35.09% of glucose uptake with ration treatment R4, while for R3, R2 and R1, the values were 23.96%, 43.28% and 23.33%, respectively. The remaining glucose taken up by the mammary cells would be utilized for other processes like oxidative catabolism, supply of carbon skeleton for the synthesis of TG-glycerol, AAs and other milk components. The low glucose utilization for lactose synthesis (40 to 60%) has been viewed by Chaiyabutr et al. (2000) as caused by the inhibition of lactose synthesis at the stage of UDP-galactose from glucose-6-phosphate as was observed with Friesian×

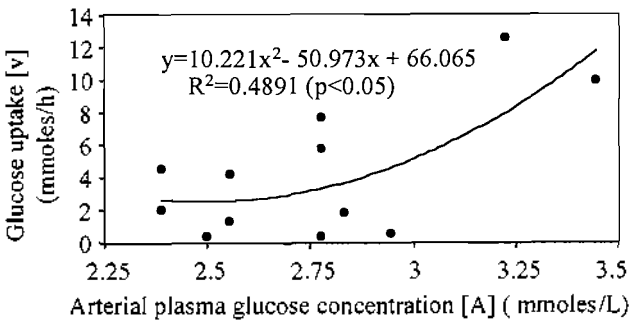

Figure 1. Quadratic relationship between mammary glucose uptake [v] and arterial blood plasma glucose concentration [A] of first lactation Bali cows

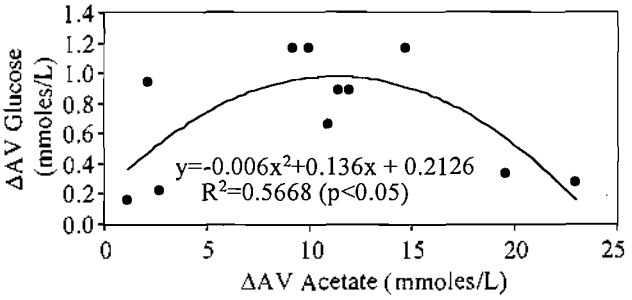

Figure 2. Quadratic relationship between mammary AV difference of glucose (⊿AV Glucose) and AV difference of acetate (⊿AV Acetate) of first lactation Bali cows

local Thailand cattle crossbreds, fed fermented rice-straw urea mixture plus concentrate.

In conclusion, even though highest milk yield was obtained with cows fed a grass-legume diet plus concentrate and Zn-diacetate supplement (Sukarini et al., 2000), the Bali cow would still have an opportunity to reach even higher yields than was obtained from the present experiment, if glucose uptake by the mammary cells for lactose synthesis could be improved.

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