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Glucose in Ruminants: A Review

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The role of glucose as an essential nutrient has been evaluated in various ways. Assays with *in vitro* systems, usually carried out near optimal conditions, provide information about which organ or tissue is capable of metabolizing glucose under normal condition, and the adaptive effect on enzyme activities caused by diet, hormones, or specific metabolites. In the intact animal, metabolism is regulated by homeostatic mechanisms including nervous and hormonal control. Furthermore, there are functional relationships between different organs and tissues in the body which are lacking in *in vitro* systems. Information on glucose metabolism in the intact animal has depended on changes in blood glucose concentration as an index of e.g. the glucogenic behaviour of precursors, although this type of information is ambiguous since blood concentration of metabolites or substrates at any time is the net result of the compound's release and utilization. Special attention will be given to data on transfer quotient (TQ) from precursor into product (glucose) which demonstrate the high glucogenic capacity of our indigenous lactating and late-pregnant ruminants.

INTRODUCTION

Research over the last 40 yrs has resulted in several major advancements in knowledge regarding the nutritional role of glucose in ruminants. The topic has been reviewed by a number of authoritative investigators in the field, namely Boekholt (1976), Bergman (1983a, 1983b, 1990), Madsen (1983a, 1983b, 1983c), Preston and Leng (1987), Brockman (1993), summarizing the knowledge of glucose metabolism and providing also access to earlier literature. The present paper refers to those work as source of information on the topic, highlighting a few important aspects and some findings.

Glucose plays a key role in the metabolism of the animal body. It is an essential source of energy for the maintenance of many tissues, e.g. the nervous system, red blood cells, the placenta and the mammary gland. It also serves as a precursor for the biosynthesis of essential cell components. Glucose oxidation is necessary for the formation of most of the reduced co-factor NADP for synthesis of long-chain fatty acids, and the synthesis of milk components. Due to the pre-gastric fermentation of feeds, little glucose is absorbed from the digestive tract of the ruminant, however, glucose is equally important as a metabolite as for all mammals. In the ruminant, the major portion of glucose must be synthesized by the process of gluconeogenesis. This process is accelerated when the demand for glucose is raised, as with the onset of lactation and in the later stages of pregnancy. Impaired gluconeogenesis, a shortage of carbohydrate precursors, or both, are believed to contribute to various metabolic disorders frequently seen in ruminants, such as acetonemia in dairy cows and pregnancy toxemia in sheep.

Information in the present presentation will be given on methodology, glucose supply, glucose needs and utilization, and touching a little on gluconeogenesis from propionate. Gluconeogenesis from amino acids is not covered here.

METHODOLOGY

Following biochemical studies with *in vitro* systems conducted in the thirties onward and studies with intact animal relying on changes in blood glucose concentration data, a major advance has been in efforts in obtaining quantitative estimates of the rate of glucose metabolism using principally two techniques: isotope dilution and arteriovenous catheterization. The techniques may be combined and two or more isotopes may be used simultaneously. The effect of supplying substrates as precursors, e.g. those arising from ruminal fermentation or through intragastric and intravenous infusion, may also be estimated from data on absorption by the splanchnic bed or ruminal glucose precursor production rates. The techniques may also be used to estimate glucose utilization by organs and tissue, e.g. liver, hind-limb muscle, mammary gland, portal drained viscera, placenta.

Tracer Techniques

The tracer dilution technique depends upon the principle of isotope dilution, i.e. the concentration of tracer at any time (specific radioactivity, percent excess abundance) that is inversely related to the mass of tracee homogeneously mixed with the tracer. It is the prime technique for measuring the exchangeable masses/volumes of components/compartments of living animals. The conventional isotope model for the determination of hepatic glucose was described by Steele (1959). The original approach was based on the use of glucose-U-^{14}C. Katz & Dunn (1967) demonstrated the advantages of using glucose-6-^{3}H, in that tritium does not materially recycle via the Cori cycle and nearly all of the tritium is released into water. With glucose-3-^{3}H, the loss of tritium through the Cori cycle is even more complete, but some tritium could be lost in the liver pentose cycle. With glucose-2-^{3}H, there is extensive detritiation at the hexose-P level and this phenomena is advantageously used to measure real hepatic glucose production or uptake (Rognstad 1994). The main difficulty in the tracer dilution technique is to ensure uniform mixing of tracer and tracee. Data from serial samples provide valuable information on the kinetics of the mixing process.

The single injection technique involves the administration of a single dose of tracer and allowed to mix rapidly. From the exponential decline with time of the tracer concentration in the tracee, glucose kinetics are estimated. An alternative technique is the constant infusion technique, that involves primed continuous infusion of labelled glucose solution into the jugular vein and after reaching steady state condition of isotope concentration in body fluid glucose, serial blood samples are withdrawn (Sastradipradja 1970). Glucose flux is calculated by dividing the rate of labelled glucose infused by the plateau label concentration of blood glucose. A similar primed continuous infusion technique using bicarbonate-^{14}C, called the carbondioxide entry rate technique (CERT) is used to measure CO_2 production rate, hence, heat production of the intact animal (Corbett *et al.* 1971). The advantage of CERT is the ability of simultaneously measuring glucose kinetics by administering glucose-^{3}H at the same time with the bicarbonate label (Sastradipradja 1992). Another advantage of CERT is the ability to estimate gluconeogenesis from carbon transfer involving CO_2 fixation into the glucose pool. The rationale involves using the transfer quotient (TQ) between CO_2 and glucose to assess the extent of CO_2 fixation in gluconeogenesis (GNG) from precursors like propionate and lactate. The TQ in question is calculated as the ratio between the plateau specific activities (per at. C) of ^{14}C (or % excess abundance with ^{13}C label) in glucose (product) and in bicarbonate (precursor). The maximum value of this TQ is 16%. The rate of GNG involving CO_2 fixation is found by multiplying the value of the glucose flux by the TQ times six.

Absorption Measurements

Many metabolic peculiarities of ruminants stem from the viscera, that is a group of organs whose blood supply drains into the portal vein. Quantification of nutrient in the splanchnic region is useful to understand the metabolism in this region. A study on this aspect in small ruminants by the arteriovenous difference technique (Katz & Bergman 1969) involves surgical procedures to obtain samples from the mesenteric, portal and hepatic veins and of any artery (Astuti 1995). Portal blood flow was measured in anesthetized animals (with xylapex 0.05 ml/kg liveweight, followed by intra muscular injection of ketalar 0.11 ml/kg liveweight) using ^{3}H labeled para-aminohippuric acid (PAH-^{3}H), while lidocain (1 ml/animal) was applied locally on the site of incision. The primer dose of PAH-^{3}H of 5.75 μCi was delivered in one minute via the jugular vein followed by continuous infusion at 0.6 Ci/min into the right mesenteric vein. After around two hours of infusion, blood samples were collected for analysis of blood metabolites including glucose and VFA's. Metabolism and absorption rates of nutrients could be calculated using the Fick principle. The technique is invasive, however, that needs appropriate skills to perform.

Glucose uptake and utilization by the mammary gland measurements needed are on:

Mammary Blood Flow (MBF)

This physiological parameter is essential in basic trials for the calculation of nutrient utilization in lactating animals estimated using a variety of techniques. For a special purpose, the indirect technique employing the Fick principle, used initially total N in milk, arterial and mammary venous blood for the calculations (Astuti 1995). Later measurements applied a technique according to Cant *et al.* (1993) using A-V difference of phenylalanine and tyrosine, and their contents in milk protein.

Thus,

MBF (liters per hour) = $\{(FY_B \times .965) + FY_F\}/\{FY_{A\text{-}V}\}$,

where:

FY_B = Phe + Tyr output in milk protein (moles per hour),
FY_F = free milk Phe + Tyr (moles per hour), and
$FY_{A\text{-}V}$ = Phe + Tyr A-V difference (moles per hour).

Metabolite Uptake

Data on blood and plasma metabolite concentrations is needed and using the Fick principle, extraction and uptake by the mammary gland can be calculated. Metabolites of interest are oxygen, glucose, acetate, betahydroxy-butyrate (BHBA), lactate, glycerol, triglycerides (TG), other lipids, amino-acids. O_2 uptake and CO_2 production provide the basis for estimating substrate oxidation.

Feasible techniques to measure ruminal fermentation production rates would be an alternative approach to measure the nutrients' supply to the animal. *In vitro* techniques measuring microbial protein synthesis, rate of formation of end-products (VFA's), rate of release of NH_3, gas production etc. simulate *in vivo* conditions. There are direct *in vivo* isotopic techniques for measuring VFA production rates. Of interest is the non-radioisotopic technique referred to as the zero-time *in vitro* method (Whitelaw *et al.* 1970), where a sample of rumen content is taken and sub-samples incubated *in vitro* under anaerobic conditions. The rate of production of individual and total VFA is calculated from the increments in acid concentration obtained by incubating the subsamples at different periods and extrapolating back to zero time incubation to give the rate of VFA production per unit volume at the time the sample was removed. Equations for performing the calculations are given by Whitelaw *et al.* (1970). The rumen volume should be known in order to calculate total ruminal production.

GLUCOSE SUPPLY

The ruminant is in a disadvantageous position as compared to monogastric animals with regard to glucose supply, because only limited amounts of glucose and/or glucose polymeres reach the intestines:

1. Little escapes ruminal fermentation and reached unchanged the duodenum. The amount may vary widely, but seldom exceed 25% of that supplied.

2. Another minor portion of feed carbohydrates will be transformed into microbial polysaccharides and may reach the duodenum as stored microbial carbohydrate.
3. Most undergo fermentation in the rumen and hydrolyzed into acetic, propionic and butyric acids.

The availability of glucose or its precursors depends primarily on the amount of glucose that is absorbed from the intestines and on the amount of propionic acids produced in the rumen.

The Amount of α-Glucose Polymeres

Carbohydrate recovered in the duodenum is usually indicated as starch or α-glucose polymeres. Results of various measurements vary depending on the methods of analysis employed or origin of the samples from abomasum or duodenum via re-entrant cannulas and the indicators used, variation due to animal species and the structure and ration components.

Weller & Gray (1954) estimated that most of the starch in the forestomachs of sheep existed in the liquid phase of the digesta, mostly contained in rumen protozoa. A maximum of 7.8 g starch was found passing through the abomasum when the diet contained 148 g starch. With 20 - 40 g starch in the ration about 10% reached the abomasum, while with a ration containing 2.9 g starch the amount became 1.1 g. Higher percentages passing the duodenum was reported by Karr *et al.* (1966) using young oxen fed ground rations composed mainly of lucerne hay, corn and soyshot containing 19 - 64% starch. The amount of starch passing the duodenum (16 - 38%) increased with increasing percentage of starch in the ration. The authors assumed that the digestive capacity of the small intestine is not unlimited because appreciable amounts of starch reached the ilium. This is in agreement with the results of ϕrskov & Fraser (1968) and Little *et al.* (1968). Heat treated cassava fed to growing sheep, water buffaloes and lactating goats lowered starch passage into the duodenum as compared to untreated cassava (Sofjan 1983, Manik & Sastradipradja 1989).

Production from Propionic Acid

From the VFA's produced in the rumen, only propionic acid contributes to glucose synthesis in the body. From one molecule of hexose: 2 molecules of acetic acid, 2 molecules of propionic acid or 1 molecule of butyric acid can be formed. Based on a molar ratio of 60:24:16, the VFA's are produced from 30 + 12 + 16 molecules of hexose. According to this calculation 12 out of 58 hexose molecules are transformed into propionic acid, that is approximately 20%. The remaining 80% will not contribute to body glucose supply. Boekholt (1976) reported the following calculation of the quantity of propionic acid that can be produced by a lactating cow of different production levels (Tables 1 & 2). The calculations were for a 500 kg cow producing 10, 20, and 30 kg milk per day, ME maintenance = 481.3 $W^{0.75}$ kJ and requirement for production 5.23 MJ/kg milk. From the remaining ME, at least 150 g, 280 g, and 410 g respectively, will reach the duodenum as starch that escaped fermentation and part incorporated as microbial polysaccharides. After substraction, the remaining ME resides in the VFA's, assuming that the average heat content of VFA is 18.41 kJ/g, it is easy to calculate how much propionic acid is formed.

Table 1. Calculation of the quantity of propionic acid which can be produced by a lactating cow of different production levels (Boekholt 1976).

Milk production, kg/day		10	20	30
ME maintenance requirement	MJ/d	50.22	50.22	50.22
production requirement	MJ/d	52.31	104.62	156.93
ME total requirement	MJ/d	102.53	154.84	207.15
Ruminal heat production 10%	MJ/d	10.25	15.48	20.72
Energy in protein and fat 20%	MJ/d	20.51	30.97	41.43
Energy in α-glucose polymeres	MJ/d	2.72	5.02	7.11
Energy in fatty acids	MJ/d	69.05	103.37	137.90
Fatty acids (18.41kJ/g)	g/d	3750.00	5615.00	7490.00
Propionic acid 26%	g/d	975.00	1460.00	1945.00

Tabel 2. Heat of combustion of fatty acids.

	molar (%)	MW	kJ/mol	weight (%)	kJ/g
	(a)	(b)	(c)	(ab/Σab)	(c/b)
Acetic acid	60	60	874.67	53	14.56
Propionic acid	24	74	1535.90	26	20.76
Butiric acid	16	88	2192.94	21	24.69

Manik & Sastradipradja (1989) measured the TQ of carbon from bicarbonate into glucose, which could be used as an index of gluconeogenesis involving fixation of CO_2 reactions such as from lactate, propionate, pyruvate and alanine (Table 3). When available, propionate has preference in endogenous glucose production over the other compounds (Brockman 1993), thus, by adding steam cooked cassava in the concentrate supplement for lactating and non-lactating does instead of raw cassava, 70 - 80% of the glucose flux would have arisen from propionate. A similar approach to study the effect of feeding King grass silage with chicken manure on the metabolism and glucose production rates of lactating goats demonstrated propionate and other 3-carbon compounds to be the main precursor, of endogenous glucose production (Kiranadi *et al.* 1994).

Table 3. Glucose metabolism of growing female and lactating Etawah crossbreed goats receiving a supplement diet containing steamcooked cassava as compared to unprocessed cassava (Manik & Sastradipradja 1989).

Parameter		Sole grass diet	Raw cassava	Steamcooked cassava
ME (MJ/day)	G	2.49	3.26	4.24
	L	2.58	3.59	4.31
HP (MJ/day)	G	3.05	2.76	4.65
	L	2.31	2.63	3.69
(MJ/day)	G	0.03	0.07	0.10
	L	0.06	0.10	0.13
Glu. Flux (mg/min)	G	17.70	16.10	31.40
	L	14.20	15.00	29.20
TQ CO_2 - glucose (%)	G	9.10	6.20	13.20
	L	6.00	7.40	12.90
GNG inv.CO_2 fixation (mg/min)	G	9.70	5.90	24.90
	L	4.90	6.30	22.80

G = growing goats, L = lactating does

Ruminal propionate production rates in growing female goats fed isoenergetic diets (18.6 MJ/kg DM) but different CP levels (18.5 - 14.0% DM), were estimated by Putra *et al.* (1997) who found values from 0.425 to 0.615 moles/day corresponding to a supply of 4.13 to 5.98 g glucose for 100% conversion.

Two moles propionate (148 g) would produce 1 mol glucose (180 g), while 1 mol α-glucosepolymeres (162 x n) after hydrolysis would form 180 x n glucose. Thompson *et al.* (1975) infused propionate labeled with ^{14}C and glucose-^{3}H to get data on glucose synthesis in lactating cows. Their findings showed that not all propionate was used for glucose synthesis, but only 60% was transformed. Thus, total glucose supply for a 500 kg cow would be 875, 1375 and 1870 g of glucose for daily production levels of 10, 20, and 30 kg milk, respectively (Table 4).

GLUCOSE REQUIREMENT

The need for glucose is usually estimated from glucose-isotope dilution in blood, with the quotient between the rate of infusion and the specific activity as the basis for calculation of supply to the blood through various pathways: (1) absorption from the gastrointestinal tract, (2) glycogenolysis in liver and muscle and, (3) gluconeogenesis. Under a steady state situation, the incoming glucose into the body glucose pool equals the amount leaving the pool, hence, many terminologies have been given to name the flow: glucose flux, glucose entry rate, glucose transfer rate, turnover rate, irreversible loss of glucose.

Glucose for Maintenance

In ruminants, absorbed glucose supplies just a part of the glucose requirements. Glycogenolysis seems to add only to meet the requirement, on the other hand, gluconeogenesis depends not only on requirement, but depends also on the availability of glucose precursors. Glucose requirements for maintenance are obtained from data with fasting animals, and under 24 - 96 h fast, a relationship for ruminants was found between glucose flux (G) and body weight (W). From data appearing in the literature Ballard *et al.* (1969) estimated log G = 0.456 + 0.807 log W, thus, for a 350 kg cow, glucose flux would be 323 mg/min or 465g/day.

Table 5 lists data on entry rates of a number of ruminant species fasting for at least 24 h, including data with indigenous goat and sheep for comparison. Taken the value for fasting as approximating the maintenance requirement (around 3.3 mg/min/kg$^{0.75}$), the value for the 350 kg cow would be 385 g/day. Looking at the data of the PE goats, the estimated glucose "maintenance" requirement of 3.3 g/d may not apply for tropical indigenous ruminants. On a low level of feed intake, glucose flux of the PE goat would measure less than 1 mg/min/kg$^{0.75}$.

Glucose for the Synthesis of Milk Lactose and Glycerol

Apart for maintenance, glucose in lactating animals is needed for lactose synthesis. For synthesis of 1 mol lactose (342 g), about 2 mol glucose (360 g) is needed and to produce milk with a lactose content of 4.8%, 50.5 g glucose is needed per kg milk. If glucose (or precursors) requirement is 12% of the quantity of fat synthesized, then milk with a fat content of 4% requires 40 x 0.12 = 4.8 g glucose per kg milk. This would be at maximum, since milk fat is also derived from feed. Total glucose requirement for milk production is about 55 g per kg milk. In addition, the glycerol required for the synthesis of milk fat originates from glucose precursors.

Glucose for the Synthesis of Fatty Acids

Fatty acid synthesis in mammary tissue occurs in the cytoplasma, initiated by the formation of malonyl-CoA.

$$\text{acetylCoA} + CO_2 + \text{ATP} \rightarrow \text{malonylCoa} + \text{ADP} + \text{P}$$
$$n\ \text{malonylCoA} + \text{acetylCoA} + 2n\ \text{NADPH} \rightarrow$$
$$CH_3.\ CH_2.(CH_2)_{n-1}.CH_2.\text{CO-CoA} + 2n\ \text{NADP} + nCO_2$$

Cytoplasmic fat synthesis requires NADPH as the reducing co-factor, but because there is no cytoplasmic transhydrogenation enzymes to catalyze the transfer of H^+ from NADH to NADP (not like mitochondria), NADPH must be synthesized by other biochemical reactions. Among the possibilities of obtaining cytoplasmic NADPH in ruminants, the glucose metabolism via the phosphogluconate pathway (or pentose phosphate cycle) would provide the majority of the NADPH needed for synthesis of longchain fatty acids in mammary tissues. Up to 50% of the NADPH requirement is met through other reactions e.g. dehydrogenation of isocitrate to 2-oxoglutarate (Baumann & Davis 1975). If all NADPH is produced from glucose, calculations show that for the synthesis of 1 kg milk fat, 49 moles NADPH are needed from 4.06 moles glucose or 730 g. Maximum glucose required for fatty acid synthesis is 73% of the amount of fat. It is therefore apparent that the manner in which NADPH is produced would have great influence on the glucose requirement for milk production.

Thus, glucose requirement for maintenance and milk production (4.8% lactose and 4% fat) can be quantified based on the assumption that 50% of the NADPH requirement would be provided by glucose. Glucose requirement for carbon of lactose and glycerol, and reducing co-factors would be respectively 55.3 and 14.5 g per kg milk or a total of

Table 4. Calculated total glucose supply for a 500 kg lactating cow, if 100% or 60% of propionate is converted into glucose (Boekholt 1976).

Milk Production, kg	100%			60%		
	10	20	30	10	20	30
Glucose from propionate, g	1185	1775	2365	710	1065	1420
Glucose from α-glu.polymeres, g	165	310	450	165	310	450
Total glucose supply, g	1350	2085	2815	875	1375	1870

Table 5. Glucose flux of female sheep, goats and cattle.

Hours fasting or physio. state	Number	Bodyweight, kg	Glucose flux, mg/min/kg$^{0.75}$	References
Sheep				Boekholt (1976)
24	13	34	3.55	
48	2	40	2.98	
24		35	3.96	
48		35	3.06	
96	2	44	3.10	
24		45	3.05	
120	2	49	2.50	
24	3	30	4.20	
24	2	31	2.80	
24 - 30	4	35	3.80	
average for temperate sheep			3.30	
Cattle				
48 - 120, lactating	4	465	4.94	Boekholt (1976)
lactating, before morning feed	6	472	7.83	Anand (1969)
Goat, toggenburg				Sastradipradja (1970)
lactating, before morning feed	11	49	5.28	
Sheep, javanese thin tail				Sastradipradja *et al.* (1991, 1994b)
non-pregnant, fed	8	av. 18	4.98	
pregnant, fed	13	av. 18	6.97	
Goat, PE				
non-pregnant, fed	9	av. 25	2.24	Sastradipradja *et al.* (1994b)
pregnant, fed	18	av. 25	2.85	
non-lact.,grass(+raw cassava)	12	av. 16	2.09	Manik & Sastradipradja (1989)
non-lact., grass(+cooked ")	6	av. 16	3.93	
lact., grass(+ raw cassava)	18	av. 16	1.83	
lact., grass (+ cooked cassava)	9	av. 16	3.65	
lactating, fed with supplement	20	av. 21.5	3.28	Sastradipradja *et al.* (1994a)
lactating, King grass	5	av. 20	2.85	Kiranadi *et al.* (1994)
lactating, King grass silage	10	av. 20	4.69	
growing, 4.47 MJ/d ME intake	4	7.75 kg$^{0.75}$	2.71	Astuti *et al.* (1997)
growing, 3.20 MJ/d ME intake	12	7.17 kg$^{0.75}$	0.94	
growing, 2.40 MJ/d ME intake	4	6.48 kg$^{0.75}$	0.72	
lactating, 9.26 MJ/d ME intake	4	10.96 kg$^{0.75}$	2.69	
lactating, 8.85 MJ/d ME intake	4	11.34 kg$^{0.75}$	2.13	
lactating, 7.10 MJ/d ME intake	4	10.45 kg$^{0.75}$	1.38	

70 g glucose per kg milk or 1200, 1900, and 2600 g glucose for milk production of 10, 20, and 30 kg, respectively.

The relative contributions of the two pathways of NADPH generation should consider other factors. Preston & Leng (1987) stated that account should be taken for a balanced supply of ATP and NADPH in tissues which specializes in only fat synthesis. Furthermore, the evidence from growth studies, that strongly suggests glucose as a limiting nutrient, may guide our reasoning of accepting the contribution of both pathways to optimize acetate utilization as an energy and substrate source for fat synthesis and minimize the need for glucose, particularly if blood glucose is low. Table 5 presents metabolic measurements with indigenous PE goats. It is of interest to note that the glucose flux in this animal breed even under high physiological demands of production being compensated by good feeding, is low and barely reaching the value for the temperate sheep breeds under fasting condition. Except for the Javanese thin tail sheep, no information is available for other indigenous sheep breeds. It would be reasonable to expect that low levels of glucose metabolism is applicable for other tropical indigenous breeds like Bali cattle and buffaloes which are used for draught that require high energy generation. Preliminary data of Bali cows ($\pm$ 350 kg BW) corroborate with this notion, with glucose flux values of (231 $\pm$ 74) mg/min (n=2) for fasting, (267 $\pm$ 160) mg/min (n=8) for non-fasting non-lactating, and (336 $\pm$ 169) mg/min (n=8) for non-fasting lactating cows (Sukarini & Sastradipradja unpublished data). It is in the above light of knowledge that we must see the implication of low glucose metabolism of indigenous tropical ruminants. For illustration, data on metabolic performance of lactating PE goats (Astuti 1995) is presented (Table 6).

Glucose for Work

In many developing countries, female large ruminants replace male animals as draught animal power, because, female animals are generally raised as they produce offspring, hence, being given priority for the available feed. Work requires ATP which is predominantly supplied through oxidation of fatty acids (Bird *et al.* 1981). Glucose oxidation may have quantitative importance in intensive working animals as an increase could reached 50% above resting. Mahardika *et al.* (1997) reported results of studies with working swamp buffaloes fed on a sole grass diet subjected to 1 - 3 hr work loads. It was found that fat deposition was absent in all working animals, but protein retention was only negative in animals working 3 hours. The data revealed that intakes failed to match output resulting in the utilization of body reserves. Both fat and protein were

Table 6. Metabolic data of PE lactating goats fed three levels of diets (Astuti 1995).

Parameter	Diet I	Diet II	Diet III	Sign. P level
DM intake, g	864 *ad lib.*	764	620	-
Energy consumption, MJ/d	15.98	14.07	11.38	-
ME intake, MJ/d	9.26a	8.85a	7.10b	<0.01
MBS, $kg^{0.75}$	10.96	11.34	10.45	NS
Post absorption metabolites				
Portal VFA absorption, mM/min	16.7a	12.8ab	7.7b	<0.05
Blood glucose, mg/100 ml	104.8	99.5	99.75	NS
Glucose pool, g	3.30	2.25	1.96	<0.01
Glucose flux, mg/min	29.43	24.20	14.46	NS
TQ CO_2 to glucose, %	14.73	13.63	14.75	NS
GNG inv. CO_2 fixation, mg/min	26.09	19.90	12.75	NS
Absorption by mammae *				
Glucose absorption, mg/min	54.22a	36.39ab	22.44b	<0.01
Total VFA absorption, mg/min	54.34a	47.35ab	28.01b	<005
TG absorption, mg/min	67.79a	45.03b	26.24c	<0.01
Milk				
Milk production, g/d	658a	555ab	368b	<0.01
Energy in daily milk, MJ/d	2.50a	2.08a	1.21b	<0.01
Milk lactose, g/d	34.46a	27.12ab	16.6b	<0.01
Milk fat, g/d	35.78a	26.69a	13.82b	<0.01
Milk protein, g/d	23.56ab	24.30a	15.49b	<0.05

* mammary blood flow estimated to be 500 ml per ml milk produced.

utilized by the working animals as energy source. An element in the regulation of energy exchange is the mechanism that regulates body composition, and the deposition of fat appeared to be inversely related to the general activity of the animal. Cortisol has an effect on gluconeogenesis from protein through provision of substrates in the Kreb's cycle, thus, supporting muscular activity.

The situation in working females means competition for glucose toward milk production. The availability of glucose and fatty acids is likely the primary limitation to milk production of the working cow and feed supplements must be given to ensure sustained milk yield. A similar rationale is the situation of the pregnant working cow. Preston & Leng (1987) suggested that a working pregnant animal would benefit given a source of glucogenic energy in the form of bypass protein or starch. To restore body fat reserves in the working swamp buffaloes glucose or its precursors should be provided with the feed.

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